

Naval Research Laboratory

Stennis Space Center, MS 39529-5004



NRL/MR/7333--96-7725

Fungal Contamination of H-53 Aircraft

DENNIS M. LAVOIE
BRENDA J. LITTLE

*Ocean Sciences Branch
Oceanography Division*

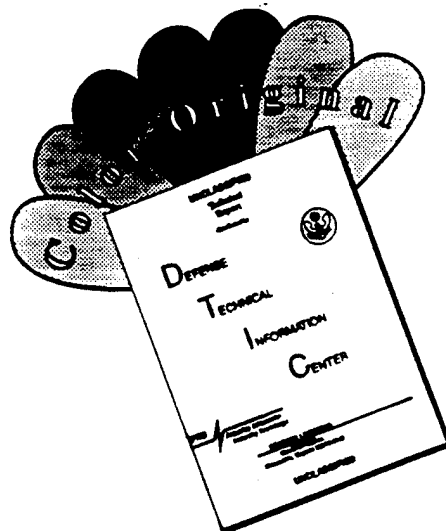
March 15, 1996

19960419 171

Approved for public release; distribution unlimited.

DTIC QUALITY INSPECTED 1

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF COLOR PAGES WHICH DO NOT REPRODUCE LEGIBLY ON BLACK AND WHITE MICROFICHE.

REPORT DOCUMENTATION PAGE

Form Approved
OBM No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE March 15, 1996		3. REPORT TYPE AND DATES COVERED Final	
4. TITLE AND SUBTITLE Fungal Contamination of H-53 Aircraft				5. FUNDING NUMBERS Job Order No. 573594500 Program Element No. 0602234N Project No. Task No. R3452, R3480 Accession No. DN16-3744	
6. AUTHOR(S) Dennis M. Lavoie and Brenda J. Little					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Research Laboratory Oceanography Division Stennis Space Center, MS 39529-5004				8. PERFORMING ORGANIZATION REPORT NUMBER NRL/MR/7333--96-7725	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research 800 North Quincy Street Arlington, VA 22217-5660				10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited.				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Fungal contamination of aircraft interior surfaces has the potential to influence corrosion of airframes, cause acute or chronic health problems in crews, and causes excessive maintenance efforts. Nine genera of microfungi were isolated from H-53 aircraft in various stages of the depot maintenance cycle at Naval Aviation Depot, Cherry Point, NC, in October 1995. The results of this study support previous studies that demonstrated a potential for corrosion of unprotected structural aluminum due to fungal and bacterial growth. The most immediate concern, however, appears to be cleaning maintenance procedures because of the waste of manpower and the potential for corrosion damage from unauthorized procedures, particularly the use of bleach to eliminate "mildew" stains left behind after physical removal of fungal growth. It is not known if mildew staining is a problem specific to the older, lacquer paint or if it occurs on the newer, polyester urethane (polyurethane 36321) paints as well. At least one of the fungi isolated from H-53 interior surfaces is known to discolor paint. No physical degradation of polyurethane and lacquer coatings was observed in this short term study, and standard cleaning procedures with alcohol appeared to remove surface fungi effectively. Recommendations for immediate actions are: (1) continue current procedures to disinfect and clean painted surfaces with possible minor changes, (2) repaint interiors of aircraft undergoing depot maintenance with gloss polyurethane to make cleaning easier, (3) identify additives/inhibitors already proven to be effective by the paint industry for immediate use in problem areas such as bilges and other areas difficult to reach during normal maintenance, and (4) conduct research to further evaluate specific inhibitors of staining fungi. Recommendations for long term studies are: (1) evaluate potential for bacterial- and fungal-influenced corrosion in structural aluminum, (2) investigate inhibitors of fungi that degrade polyurethane coatings, and (3) evaluate so-called "smart" technology options.					
14. SUBJECT TERMS biodeterioration				15. NUMBER OF PAGES 32	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified		18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified		19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	
				20. LIMITATION OF ABSTRACT SAR	

INTRODUCTION

The following final report concludes the preliminary study of microbial agents responsible for discoloration and growth on interior surfaces of H-46 and H-53 rotary-wing aircraft. Fungal contamination is reported to require many man-hours of cleaning maintenance in the field. In addition to the waste of manpower and the cost of supplies, drastic, unauthorized cleaning solutions are sometimes employed that may attack the structural integrity of the airframe. Additional concerns include possible degradation or breaching of protective surface coatings by the causative microorganisms with resulting corrosion, and possible adverse physiological effects (nausea, allergenic sensitivity) for the crew that may impair their performance.

The **objectives** of the project were to:

- (1) survey aircraft to determine specific problem areas and isolate/identify microorganisms growing on interior surfaces;
- (2) demonstrate potential for biodegradation and microbiologically influenced corrosion (MIC) on typical aircraft surfaces using isolated microorganisms;
- (3) propose and test procedures for immediate decontamination;
- (4) propose and test biocides/inhibitors to prevent/retard growth, biodeterioration, and corrosion.

The **approach** was a "quick look" study of aircraft available at Marine Corps Air Station (MCAS) and Naval Aviation Depot (NADP), Cherry Point, NC, to collect samples from the aircraft, and to evaluate corrosion problems related to the microorganisms isolated from the samples. Tests included: (a) exposures of painted aluminum coupons to fungal growth; (b) microscopic examination of exposed coupon surfaces for evidence and characterization of microbial attack; (c) electrochemical tests on bare aluminum coupons to determine aggressiveness and mode of attack.

METHODS

General Descriptions of Sampled Aircraft

Swabs and solid samples were collected from cargo bay interiors of aircraft available at MCAS and NADP, Cherry Point, NC on 11 October 1995. The following H-53 aircraft were available:

(1) Two aircraft in salvage storage, model CH-53A, #152403 and #153311. The open hatches of these aircraft had been sealed when first placed in storage but were now open to outside air. Fungal growth was found on virtually all interior surfaces, including primer-coated and polyurethane-coated aluminum and fiberglass structural members, caulking, synthetic fabrics, and wiring.

(2) One aircraft on line, waiting to be moved into hangar for depot overhaul, Model MH-53J (Air Force), # not noted. This aircraft was parked with the rear hatch open. Interior surfaces were relatively clean except for a painted bulkhead behind an avionics rack position. The bulkhead was apparently difficult to reach for cleaning and was subjected to elevated temperatures from the electronics. This area was encrusted with a thick, black growth that was difficult to remove. There was a noticeable oil film on most surfaces.

(3) Two aircraft in depot hangar. Model RH-53D, #158751, had been partially cleaned. Spotty black growths were attached to damp bilge surfaces. Model CH-53D, # 157176, had not been cleaned and had extensive black growth on many surfaces. Surfaces were oily.

Sample Collection

Sample descriptions are provided in Table 1. Samples were collected for bacteriological analysis, isolation/identification of fungi, and microscopic examination of contaminated surfaces, including aluminum sections, fiberglass, Velcro® strips, and caulking.

For bacteriological analysis, MICKIT[®] proprietary media were used to screen solid or liquid samples for bacteria known to be involved in MIC and to approximate their numbers through serial dilutions. Areas of obvious contamination were wiped with sterile cotton-tipped swabs that were subsequently placed in vials of sterile buffer solution. Each vial was thoroughly shaken to remove cells adhering to the cotton, and 1-ml aliquots of the resulting suspension were withdrawn and injected into prepared vials containing growth media specific for four groups of microorganisms: sulfate-reducing bacteria (SRB), acid-producing bacteria, general anaerobic bacteria, and general aerobic bacteria. Vials were examined 37 days after inoculation. Positive results were either the appearance of turbidity or color change in an indicator, depending on the medium.

To screen fungi, semi-solid agar medium, potato-dextrose agar (PDA), was dispensed into special containers (Rodac[™] plates) that allow inoculation by removing the cover and pressing the agar surface against the surface being tested. Inoculated plates were incubated at room temperature until surface colonies could be discerned. Individual colonies were removed and streaked onto fresh plates of the same medium to isolate the fungi. Isolate plates were incubated at room temperature until colonies appeared, then were stored at 4°C to retard growth. Isolated microorganisms were periodically re-streaked onto PDA for maintenance. Isolates were identified by characteristic growth on Sabourauds Medium, PDA, or cornmeal agar (CMA) and by microscopic examination of spore-bearing bodies (conidia).

Environmental scanning electron microscopy (ESEM) was used to examine samples of contaminated materials collected from the helicopters and to determine the extent and nature of attack on coupon coatings. ESEM is a relatively recent innovation in scanning electron microscopy (SEM) that allows specimens to be viewed in their natural state (Danitalos, 1991). Samples may be inserted into the instrument and viewed with virtually no preparation such as dehydration, heavy metal coating, or high vacuum, which are all required for standard SEM. Some organisms, like fungi, even retain their viability (see Little et al., 1991; Collins et al., 1993; Lavoie et al., 1996).

Electrochemical Tests

Electrochemical techniques are sensitive tests for measuring corrosion. Standard electrochemical methodology requires that test metals be completely immersed in electrolyte. Such tests have shown in past work that *Cladosporium*, an ubiquitous microfungus like the ones isolated in this study, is capable of corroding 2024 aluminum alloy in the presence of chloride using oil as a nutrient source (Videla, 1985). Such conditions might be expected in wet bilge compartments of H-46 and H-53 helicopters.

To investigate the possibility of corrosion occurring under a thin film of water in humid conditions (e.g., in overhead areas), disposable filter funnels were adapted to make a novel electrochemical test cell, shown in Figure 1. Coupons were placed on PDA medium poured to a depth of about 2 cm over the membrane filter. The PDA was sterilized under a UV light in a glove box for 18 hours. Galvanic coupling between cells was obtained via a hollow plastic pedestal beneath the coupon filled with non-nutritive, KCl-saturated agar that made contact with both the bottom of the coupon and saturated KCl solution in the reservoir beneath the filter. The filter acted as a biological barrier, and the 2-cm of agar served to limit exposure of the fungus growing on the agar surface to concentrated chloride ion. A pair of cells was galvanically connected through KCl solution via their side arms. Due to small differences between the cells a small electrical potential developed, and a sensitive galvanometer attached between the two cells was used to monitor the sign and magnitude of galvanic current. After a 4-hr baseline period to determine which cell in the pair was exhibiting cathodic behavior, that cell was inoculated by smearing the agar surrounding the coupon with a cotton-tipped swap that had been rubbed on a sporulating fungal colony. The second unit was left uninoculated as a sterile control. A change by the inoculated cell to anodic behavior during the test indicated fungal-induced corrosion. Two sets of test cells were prepared, one inoculated with isolate #25A and one with isolate #23A (see Results section for description of fungal isolates). Incubation was carried out at room temperature (18°C).

Aggressiveness Tests

Coupons of 1 x 1 inch, 2024 T-6 aluminum alloy were coated with chromate primer/polyurethane and with chromate primer/lacquer by NADP, MCAS, Cherry Point, NC, following appropriate federal specifications. Coupons were exposed to selected fungi under humid conditions to test potential for paint biodegradation and metal corrosion as described below.

All coupons were scratched vertically down the center to expose bare aluminum to simulate a break in the surface coating, swabbed thoroughly with 75% ethanol, and dipped (with the scratch vertical) halfway into a solution of household bleach (5.25% sodium hypochlorite). One third of the coupons was used without further treatment, while one third of the coupons was swabbed on one side with hydraulic oil, and the remaining third was swabbed on one side with "Solution A", a lanolin-based preservative supplied by DADP. Sets of three coupons (one each of alcohol, hydraulic oil, and lanolin treatments) were then placed on PDA in Petri dishes. Agar surrounding each coupon was inoculated using a sterile cotton-tipped swab that had been rubbed on a sporulating isolate colony. The purpose was to grow a thick colony of mycelia (vegetative portion of a fungus) and conidia (reproductive bodies) around each coupon that would either overgrow the coupon or provide a source of inoculum to the coupon surface. To further encourage growth, inocula were smeared on the center of each coupon. Five fungal isolates were used as inocula: #23A *Trichoderma*, #25A *Aureobasidium*, #25B *Stemphylium*, #27 *Penicillium*, and #36 *Hormodendrum* (see Results section for description of these fungi). The resulting experimental matrix was: 5 isolates x 2 paints (polyurethane or lacquer) x 2 bleaching treatments (bleached or not) x 3 surface treatments (alcohol only, oil, or lanolin). Incubation was carried out in the dark at 2° C. The experimental design is schematized in Figure 2.

Coupons were photographed to document the extent of growth over their surfaces at 4, 8, 23, and 32 days. At 32 days, coupons were examined visually and with a binocular optical microscope and qualitatively scored for surface contamination. An 75% ethanol-dipped swab was used to clean a spot on each coupon, and coupons were then examined using ESEM for evidence of attack on the painted surfaces and for cleaning efficacy.

RESULTS

Bacteriological analysis

MICKIT® results are presented in Table 2. Small numbers of SRB were detected on the five tested surfaces. No acid-producing bacteria were detected. General anaerobic/facultatively aerobic bacteria as well as general aerobes were strongly positive on some surfaces but not others.

Microscopic examination

During sample collection, two types of growths were obvious to the unaided eye: black, oily-looking spots/masses (Figure 3) and drier-looking black spots/masses having a growth pattern radiating from the center (Figure 4). The oily colonies exhibited no conidia or mycelia and presented an impenetrable surface to ESEM analysis. The presence of microorganisms in this type of mass could not be confirmed by microscopy. On the other hand, the dry, radiating colonies were obviously fungal with recognizable conidia. Figure 5a shows fungal mycelia (masses of hyphae or individual filaments of cells) attached to a polyurethane paint surface, as well as a large amount of adhering, mostly mineral, debris. Figure 5b is a higher magnification that shows individual cells in hyphae and conidia (specialized cells containing one or more spores). When a colony was wiped off painted aluminum, the paint underneath appeared to be intact. When a similar colony growing on painted fiberglass was wiped, paint under the colony, but not surrounding it, was removed as well.

Figures 6a and b show baseline ESEM micrographs of polyurethane-coated and lacquer-coated coupon surfaces before any experimentation. At a magnification of 500x, the polyurethane coating is noticeably smoother (Figure 6a) compared to the sharp, porous surface of the lacquer (Figure 6b). The differences in relief are apparently due to different forms of silica used to "flatten" the

gloss of the coating. Fused silica is used in polyurethane while diatomaceous earth is used in lacquer. Shallow craters like the one visible in the upper middle of Figure 6a were not unusual in the polyurethane surface

Fungal Isolates

Nine genera of fungi were identified, as listed in Table 3. Appearances of fungi growing on any particular surface depends on several variables, including available nutrients, colony age, physiological state, and growth environment. Descriptions in Table 3 apply only to growth under specific culture conditions and are given in this report only for reference purposes.

All isolates are ubiquitous genera. *Aureobasidium* is particularly interesting in that its appearance in culture corresponds to the oily-appearing growths observed on some of the aircraft. The organism is known to grow on and cause discoloration of latex paints (Zabel and Terracina, 1980) Micrographs of the spore bodies used as identifying features for the isolates are shown in Appendix A.

Electrochemical Tests

Galvanometer current and polarity results are preliminary but suggest that at least two fungal isolates are capable of causing corrosion of aluminum. Figure 7a & b. As described in Methods, cells were set up such that a positive current (i.e., anodic, giving up electrons) indicated that corrosion was occurring on the coupon inoculated with fungi. The cell inoculated with isolate 23A (*Aureobasidium*) went from cathodic (negative current) to anodic (positive current) during the course of the experiment. The cell inoculated with isolate 25A (*Trichoderma*) exhibited a trend toward anodic conditions, although a transition was never actually achieved. ESEM examination of the coupons revealed the surfaces were heavily coated with oxide with little visible evidence of mycelial growth, so the exact nature of attack could not be confirmed.

Aggressiveness Tests: Painted Coupons

Aggressiveness tests were run for 30 days. Results of qualitative estimates of fungal growth on the surfaces of painted coupons are summarized in Table 4. In general, lacquer-coated coupons appeared to be more susceptible to fungal colonization than polyurethane-coated coupons, scoring a total of 46 vs. 39. Of the 5 isolates tested, #36 (*Hormodendrum*) was the most aggressive in its growth on both coatings; almost half of each coupon was covered after only 4 days exposure (data not shown). Presence of oil and lanolin seemed to slightly decrease initial colonization in these tests.

In the ESEM, fungal hyphae were well established on polyurethane surfaces. Figure 8 shows a mycelial mass with conidia, while Figure 9 shows a germinating spore. Although hyphae were firmly attached on polyurethane, there was no obvious degradation at attachment points (Figure 10). Field procedures specify swabbing with 100% isopropyl alcohol for disinfecting paint surfaces (NAVAIR 01-1A-509, T.O. 1-1-691, TM 1-1500-344-23, Aircraft Weapons Systems Cleaning and Corrosion Control). In this study, 75% ethyl alcohol was substituted as an equivalent disinfectant/cleaner. Figure 11 indicates that not all fungal structures were removed by the simulated field cleaning procedure, but remaining fungi would be expected to be non-viable due to the biocide activity of alcohol.

Fungi were present in the unbleached half of the scratches on many coupons, along with occasional corrosion products (Figure 12). Hyphae were also present on the bleached half of the scratches where corrosion was heavy (Figure 13), but corrosion was attributed to oxidation by sodium hypochlorite.

No corrosion or blistering occurred on polyurethane-coated coupons; however, a few blisters were observed on lacquer-coated coupons, mostly close to the central scratch. Although there were few signs of fungi inside the blisters (Figure 14) blister sides were covered with fungal mycelia (Figure 15).

Candidate Fungicide Additives

A compendium of biocides used in industry was published by the International Centre for Coatings Technology in May 1995 (Smith and Springle, 1995) listing 500 compounds in current use, target organisms, toxicology data, general chemical class, and specific chemical makeup, among other data. Of the 473 compounds listed as having fungicidal activity, only 24 had no human hazards listed (Table 5). This may be due to lack of data or omissions in tabulation. In an effort to the list of candidate fungicides, we contacted paint manufacturers for their recommendations. One (Deft, Inc. of Irvine, CA, a major supplier of polyester urethane paint to the military) indicated that their research had identified a compound that inhibited fungal growth for up to 20 weeks according to the fungal resistance tests specified in MIL-STD-810. Effective concentrations were as low as 0.1% (by weight) when tested in two gloss white coatings: MIL-C-85285 Type I (03W040) and MIL-C-85285 Type I (03W127A), similar in composition to the polyester urethane paint tested in this report. The fungicide (whose composition is proprietary) was considered too expensive for general use (D. Bernard, personal communication). Toxicology issues, particularly for semi-enclosed spaces such as helicopters, apparently were not considered.

DISCUSSION

Field Observations and Isolated Fungi

Fungal contamination occurred to some extent in all aircraft examined. Only two types of physical appearance were noted: an amorphous, oily-looking black growth and a drier-looking, radiating black growth. Either occurred as single spots or as encrustations. A "cottony" growth previously reported behind sound insulating mats (E. Arthur and J. Whitfield, NADP, personal communication) was not observed. Technically, the fungi isolated in this study are not mildews or molds (those organisms belong to a different fungal class and are parasitic on plants; Smith, 1955), but those terms are commonly applied to any dark fungal surface growth. Physical appearance of fungal growth on surfaces outside the laboratory is not necessarily useful for identification because of the great variations that can occur with colony age, nutrition, physiological state, and substratum, to mention some of the more important variables. The fact that at least nine genera of fungi were isolated from H-53 interior surfaces does not necessarily mean all these fungi were actively growing there. It does mean that their spores were present and that there was at least the potential for their growth.

Fungi isolated from H-53 interiors belong to the fungal Class Deuteromycetes, also known as Imperfect Fungi for the fact that only an asexual, or spore-bearing, stage has been described (*Penicillium* may also be placed in the Class Ascomycetes, depending on the species). Specific growth requirements of isolates were not addressed in this study, but they have been reported (Wang and Zabel, 1990). Many fungi grow on solid surfaces provided there is a modicum of organic material available as a carbon source. The fungal isolates are ubiquitous, so contamination of the airframes could have occurred virtually anywhere during the duty cycle. At least one of the isolated fungi, *Aureobasidium*, has been reported to cause superficial discoloration on latex paint (Zabel and Terracina, 1980).

Potential for Corrosion

Videla (1986) demonstrated that the Deuteromycete *Cladosporium* in liquid culture caused corrosion of unprotected aluminum. Conditions similar to liquid culture might be expected in bilge compartments of helicopters when water, oil, and salt air combine with fungal spores. Our purpose in designing a non-immersion electrochemical cell was to test corrosion potential under sub-aerial conditions found in other parts of the aircraft. This design is new and conclusions drawn from its use are tentative; however, preliminary data suggest that the coupon exposed to one of the isolates (#23A) became anodic, as would be predicted if more corrosion were occurring in this coupon than in the sterile control. While the second isolate (#25A) did not have as strong an effect, the general trend in this test was toward anodic conditions, suggesting that the isolate was causing corrosion.

The mechanism for such an effect was not investigated, but Videla (1986) concluded that *Cladosporium* enhanced corrosion of aluminum by producing acidic metabolic products. There is some evidence from ESEM microscopy (Figure 12) that fungi were physically associated with corrosion products formed on aluminum exposed in scratches made in the protective coating. Paint defects are to be expected during the normal duty cycle of military helicopters and may be particularly vulnerable.

The presence of SRB suggests the potential for severe corrosion problems in these aircraft. SRB could be active in anaerobic areas such as those where oxygen has been depleted by respiring organisms, such as under a heavy layer of fungal growth.

Potential for Biodeterioration

The lower colonization rate for polyurethane paint and the smoother surface suggest that this coating should be easier to keep clean than lacquer paint. In this short experiment, no surface degradation of polyurethane coatings was noted where fungal hyphae were attached. Simulated field cleaning/disinfectant procedures using 75% ethanol physically removed most fungal structures from paint surfaces, and the few remaining could be expected to be non-viable. From microscopic evidence, then, short term exposure of polyurethane paints to fungi (on the order of field maintenance cleaning periods) results in no significant physical damage, and alcohol decontamination appears to be effective. Multiple exposures to fungal contamination may have a cumulative effect in terms of visible physical damage and chemical change in the coating. Such effects can only be investigated in long term studies. Presence of hydraulic oil and lanolin preservative did not seem to significantly effect fungal growth in humid, sub-aerial conditions; however, this may not be the case in immersion conditions, such as might occur in bilges. Fungi are known to thrive at oil-water interfaces, where they produce acids that can corrode metals (Salvarezza and Videla, 1984).

It should be noted that the apparent lack of degradation of polyurethane-coated coupons during this test may be due to the short exposure time. Studies of the abilities of these fungi to stain or degrade polyurethane coatings evidently will require much longer exposure to simulate typical storage times or in-field cleaning cycles. Such tests are important. Knowledge of which fungi, if any, are capable of staining and/or breaching the integrity of polyurethane type 36321 will allow us to fashion a targeted inhibitor additive. While staining itself is not a functional problem, unauthorized in-field use of bleach to remove staining, as is reported to occur (G. Arthur and J. Whitfield, NADP, personal communication) may cause major corrosion problems in unprotected structural aluminum. In this study, corrosion in scratches to bare metal was noticeable immediately after immersion in bleach.

Over the long term, fungal degradation of coatings is a potential problem. Type 36321 is technically a polyester urethane (D. Bernard, Deft, Inc., personal communication), and this type of coating has been shown to be susceptible to fungal attack (Cook et al., 1981; Wales and Sager, 1985). Once the coating is breached, direct attack on unprotected aluminum is likely, as indicated by Videla's (1986) results and electrochemical tests conducted for this report. Longer term studies are necessary to determine the mechanism of attack on this type of coating. Formation of corrosion products and associated blistering with heavy growth of fungi on lacquer surfaces indicates a potential for biodegradation of lacquer with subsequent corrosion of aluminum. The observation that polyurethane paint disbonded from fiberglass only under fungal growth suggests that the fungi were able to degrade the performance of polyurethane coating under field conditions.

Cleaning Procedures

Alcohol cleaning appears to be an effective disinfectant procedure, based on the microscopically-observable physical removal of most fungal structures. At this time, there is no technique sufficiently better that would justify the expense of changing current field procedures. Keeping surfaces disinfected and clean is still the best prevention for these ubiquitous organisms capable of growing on a very small amount of nutrients.

Minor changes in current procedure might include using 70% isopropyl alcohol rather than 100%. The lower concentration is as good as or a better disinfectant, would be less expensive,

should be less irritating to users, and is less a fire hazard. Note that while bleach is a disinfectant, it is also a strong oxidizer that may attack organics on or in the surface being cleaned. While we know of no scientific study on the subject, it is common experience among those struggling with "mildew" on painted surfaces that a surface coating previously resistant to degradation by fungi may, in fact, be rendered more susceptible to recontamination and attack after treatment with bleach. It is not known to what extent this is a problem with the polyurethane type 36321.

Frequent detergent washes of interior surfaces is also recommended. In the normal course of duty, military helicopters collect a film of hydraulic oil on interior surfaces. Oil, in association with liquid water, is known to sustain fungal growth, and an oil film traps fungal spores as well as debris that may serve as organic substrates (see Figure 5a). Organic residue left by detergents may also serve as a growth stimulant, so they must be thoroughly rinsed with fresh water, limiting the use of this technique in situations where water use is restricted.

Finally, because cleaning is a major issue to the resolution of this problem, consideration might be given to using gloss polyurethane paint on interior surfaces. Fused silica is used to flatten paint gloss by roughening the surface and scattering reflections. The increased roughness results in more surface area for dirt, oil and fungi to collect and makes the coating more difficult to clean than one with a smooth surface.

Biocides and Inhibitors

Biocides were not explicitly tested in this study. In addition to compounds listed in the industrial database (Smith and Springle, 1995), there are proprietary compounds and compounds known only to academic researchers. After candidate compounds are identified, they must be tested to determine their extended efficacy against target organisms, compatibility with the paint carrier, and health hazards in the semi-enclosed space of a military helicopter. One source of information for candidate fungicides is the paint industry. The research conducted by Deft, Inc. is an obvious starting point for future research, which should be directed at understanding specific fungicidal mechanisms and physiological effects.

For the immediate future, 36321 paint with the Deft, Inc. additive or a similar product may be cost effective and safe if its use is confined to problem areas in H-53 and H-46 airframes, such as bilge compartments, behind avionics racks or other fixed equipment, and inaccessible areas in the overheads. This possibility should be explored immediately.

Advanced Research Issues

Total inhibition of fungi on time scales of depot maintenance cycles is not attainable at this point in coating technology, and some aircraft experience conditions that are unusually conducive to fungal growth. It would be desirable to detect heavy growth in susceptible, difficult to inspect areas before it leads to further contamination and hidden corrosion problems. Adaptation of sensors developed for biofouling control and other technologies such as fiber optics should be explored as part of a "ssmart" maintenance approach to control fungal contamination of aircraft.

SPECIFIC FINDINGS

1. Interiors of H-53 helicopters examined at Naval Aviation Depot (NADP), Cherry Point, NC were contaminated with at least 9 genera of fungi and sulfate reducing bacteria, known to be associated with corrosion in anaerobic conditions.
2. Fungal contamination was more severe in areas in the aircraft that were seldom opened (bilges) and areas that could not be reached for cleaning (e.g., behind avionics racks and tight spaces in overhead spaces). Fungi were observed on virtually every type of surface including paint, cloth, caulking, fiberglass, and wiring insulation.
3. Isolated fungi are considered non-pathogenic. All are ubiquitous and could have entered the aircraft anytime in the duty cycle. One isolate has been previously reported to cause superficial discoloration on paints.
4. Preliminary data from a novel electrochemical test cell suggests that at least some of the isolated fungi can cause or influence corrosion in unprotected 2024 T-6 aluminum used in these aircraft. Fungi were associated with corrosion of 2024 T-6 aluminum in growth tests on coupons.
5. Short-term (30-day) exposure tests on painted coupons using 5 fungal isolates did not produce microscopically-visible damage to polyester urethane (gray 36231) or lacquer (gray 36231) paint that could be attributed to fungal action.
6. Association of fungal mycelia with corrosion products, blistering on some lacquer test panels, and disbonding of polyurethane on fiberglass suggest that fungi may degrade the barrier performance of coatings on long exposure.
7. Polyurethane appeared to be more resistant to fungal colonization than lacquer. This may be attributable to the smoother polyurethane surface.
8. Cleaning fungal contamination with alcohol, as per standard procedure, was confirmed by microscopy to physically remove fungi (mycelia and spores) from painted surfaces.
9. A candidate inhibitor was identified as an additive for type 36321 polyester urethane paint. This compound has been tested by a paint manufacturer and is effective in small amounts. Issues of cost, specific uses, and safety must be addressed.

RECOMMENDATIONS

Recommendations for immediate actions concentrate on preventing fungal growth. Specific recommendations are:

- (1) continue current procedures to disinfect and clean painted surfaces. Possible minor changes in procedures include decontamination with 70% isopropanol rather than 100% (cheaper, more effective, and safer to use), and frequent detergent cleaning in-between decontamination, followed by generous rinse-down.
- (2) repaint interiors of aircraft undergoing depot maintenance with gloss polyurethane to make cleaning easier.
- (3) conduct research on the following:
 - (a) Evaluate what fungi, if any, stain polyurethane 36321;
 - (b) Determine whether a specific inhibitor is known for such fungi;
 - (c) Identify additives/inhibitors already proven to be effective in polyurethane 36321 by the paint industry for immediate use in problem areas such as bilges and other areas difficult to reach during normal maintenance.

Recommendations concentrate on: evaluating potential for bacterial- and fungal-influenced corrosion in structural aluminum, investigating microbial inhibitors, quantifying degradation of polyurethane coatings, and evaluating "smart" technology options. Studies should seek to understand mechanisms, e.g., physiological/structural effects of inhibitors on fungi.

Specific recommendations for long term studies are:

- (a) **Potential for corrosion.** Further investigations should be performed on the preliminary findings of this report and previously published reports that fungi are capable of influencing corrosion of unprotected aluminum and causing biodeterioration of polyurethane paint. The work would include using standard electrochemical techniques, refining electrochemical tests developed in this project, screening fungal types, and determining mechanisms of corrosion and biodeterioration.
- (b) **Inhibitor additives.** Fungicides should be investigated for effectiveness (particularly against staining fungi), cost, and health hazards, specifically addressing the needs of the helicopter fleet. Health hazards are a major concern, and biocides are toxic by definition. Of the hundreds of fungicides recognized by industry, only a few are considered non-hazardous. Even additives considered unfeasible for general use because of cost or safety might be used in special purpose inhibitor coatings for problem areas.
- (c) **Advanced concepts - "smart" technology.** Technology options should be investigated to develop inexpensive sensors that could be placed in inaccessible areas. Ideally, such devices could be easily monitored in the field to give early warning that fungal/bacterial contamination levels have reached unacceptable levels. Such sensors exist for biofouling applications and could be modified for this purpose. Other technology (e.g. fiber optics) should be investigated as well.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the sponsorship of this project by Dr. Lewis E. Slotter, Office of Naval Research. Mr. James Whitfield and Mr. Gib Arthur of Naval Aviation Depot, Marine Corps Air Station, Cherry Point, NC, provided invaluable field support and background information. Under supervision of Mrs. Patricia Wagner, Mr. Kevin Hart conducted the electrochemistry tests. Mr. Richard Ray performed the ESEM microscopy. Dr. Ray Scheetz of the University of Southern Mississippi identified the fungal isolates.

REFERENCES

- CAMERON, R. E., 1994. *Environmental SEM: principles and applications*. USA Microscopy and Analysis, vol. May, pages 17-19.
- COLLINS, S. P., POPE, R. K., SCHEETZ, R. W., RAY, R. I., WAGNER, P. A. & LITTLE, B. J., 1993. *Advantages of environmental scanning electron microscopy in studies of microorganisms*. Microscopy Research and Technique, vol. 25, pages 398-405.
- COOK, W. J., CAMERON, J. A., BELL, J. P. & HUANG, S. J., 1981. *Scanning electron microscopic visualization of biodegradation of polycaprolactones by fungi*. Journal of Polymer Science: Polymer Letters Edition, vol. 19, pages 159-165.
- DANILATOS, G. D., 1991. *Review and outline of environmental SEM at present*. Journal of Microscopy, vol. 162 (3), pages 391-402.
- LAVOIE, D. M., RAY, R. I. & LITTLE, B. J., in press. *Examination of Hydrated, Nonconductive Biofilms in ESEM*. In: Procedures in Electron Microscopy Module number - 10 (High Pressure SEM), Robards, A. W. & Wilson, A. J. (eds.). Centre for Cell & Tissue Research, York, UK.
- LITTLE, B. J., WAGNER, P. A., RAY, R. I., POPE, R. & SCHEETZ, R., 1991. *Biofilms: an ESEM evaluation of artifacts introduced during SEM preparation*. Journal of Industrial Microbiology, vol. 8, pages 213-222.
- SALVAREZZA, R.C. & VIDELA, H.A., 1984. *Microbiological corrosion in fuel storage tanks. Part 1: anodic behavior*. Acta Cientifica Venezuelan, vol. 35, pages 244-247.
- SMITH, A. & SPRINGLE, R. (eds.), 1995. World Guide to Industrial Biocides. Paint Research Association (International Centre for Coatings Technology), Kent, UK. 114 pages.
- SMITH, G. M., 1955. Cryptogamic Botany. Volume 1: Algae and Fungi. McGraw-Hill, NY. 546 pages.
- VIDELA, H., 1986. *The action of Cladosporium resinae growth on the electrochemical behavior of aluminum*. In: Biologically Induced Corrosion, Proceedings of the International Conference on Biologically Induced Corrosion, Dexter, S. C. (ed.). National Association of Corrosion Engineers (NACE), Houston, TX. pages 215-222.
- WALES, D. S. & SAGER, B. F., 1985. *The mechanism of polyurethane biodeterioration*. In: Biodeterioration and Biodegradation of Plastics and Polymers (Proceedings of the Autumn Meeting of the Biodeterioration Society, Occasional Publications No. 1, Seal, K. J. (eds.). Biodeterioration Society, Kew, UK. pages 56-69.
- WANG, C. J. K. & ZABEL, R. A. (eds.), 1990. Identification Manual for Fungi from Utility Poles in the Eastern United States. American Type Culture Collection (ATCC), Rockville, MD. 356 pages.
- ZABEL, R. A. & TERRACINA, F., 1980. *The role of Aureobasidium pullulans in the disfigurement of latex paints*. Developments in Industrial Microbiology, vol. 21, pages 179-190.

Table 1: Sample summary, MCAS & NADP, Cherry Point, NC, 11 Oct 95

Sample*	Airframe	Number	Location	Medium**	Sample/ Location	Remarks
1	H-53	RH53D 158751	Hanger	A : PDA press-plate	Blige	Loose, oily dirt
2	H-53	RH53D 158751	Hanger	B : NA press-plate	Blige	Loose, oily dirt
3	H-53	RH53D 158751	Hanger	D : Formaldehyde (F/a)	Blige	Oily water
4	H-53	RH53D 158751	Hanger	A : PDA press-plate	Blige, near door frame	Rosettes
5	H-53	CH53A 152403	Salvage storage	A : PDA press-plate	Side of bilge, port vertical frame	
6	H-53	CH53A 152403	Salvage storage	A : PDA press-plate	Side of bilge, port vertical frame	
7	H-53	CH53A 152403	Salvage storage	E : Dry swab, in F/a, 1.5 cm diam	Side of bilge, port vertical frame	
8	H-53	CH53A 152403	Salvage storage	F : Dry swab, in ADS, 1.5 cm diam	Side of bilge, port vertical frame	
9	H-53	CH53A 152403	Salvage storage	G : Wet swab, in ADS, 1.5 cm diam	Epoxy primer, tail before port pylon	Black, gummy rosettes
10	H-53	CH53A 152403	Salvage storage	H : Wet swab in F/a	Epoxy primer, tail before port pylon	Black, gummy rosettes
11	H-53	CH53A 152403	Salvage storage	B : NA press-plate	Tail before port pylon	Black, gummy rosettes
12	H-53	CH53A 152403	Salvage storage	A : PDA press-plate	Tail before port pylon	Black, gummy rosettes
13	H-53	CH53A 152403	Salvage storage	J : Dry swab in centrifuge tube	Tail before port pylon	Black, gummy rosettes
14	H-53	CH53A 152403	Salvage storage	K : Duct tape in centrifuge tube	Tail before port pylon	Black, gummy rosettes
15	H-53	CH53A 153311	Salvage storage	B : NA press-plate	Overhead	
16	H-53	CH53A 153311	Salvage storage	A : PDA press-plate	Overhead	
17	H-53	CH53A 153311	Salvage storage	F : Dry swab, in ADS, 1.5 cm diam	Overhead	
18	H-53	CH53A 153311	Salvage storage	E : Dry swab, in F/a, 1.5 cm diam	Overhead	
19	H-53	CH53A 153311	Salvage storage	J : Dry swab in centrifuge tube	Overhead	
20	H-53	CH53A 153311	Salvage storage	K : Fiberglass section	Overhead conduit cover	
21	H-53	CH53A 153311	Salvage storage	K : Velcro strip	Overhead	
22	H-53	CH53A 153311	Salvage storage	K : Painted Fiberglass	Overhead	
23	H-53	CH53A 153311	Salvage storage	K : Painted aluminum section	Overhead	Radiating rosettes, black
24	H-53	CH53A 153311	Salvage storage	K : Painted aluminum section	Overhead	Radiating rosettes, black
25	H-53		Salvage storage	K : Painted aluminum section	Overhead	Radiating rosettes, black
26	H-53		Salvage storage	K : Painted aluminum section	Overhead	Heavy, dry, black growth
27	H-53	MH53J (AF)	Hanger	A : PDA press-plate	Behind avionics rack location	Heavy, dry, black growth
28	H-53	MH53J (AF)	Hanger	B : NA press-plate	Behind avionics rack location	Heavy, dry, black growth
29	H-53	MH53J (AF)	Hanger	G : Wet swab, in ADS, 1.5 cm diam	Behind avionics rack location	Heavy, dry, black growth
30	H-53	MH53J (AF)	Hanger	E : Dry swab, in F/a, 1.5 cm diam	Behind avionics rack location	Heavy, dry, black growth
31	H-53	MH53J (AF)	Hanger	J : Dry swab in centrifuge tube	Behind avionics rack location	Heavy, dry, black growth
32	H-53	CH 53D	Line	B : NA press-plate	Around window, frame 103-107	Oily surface under window, heavy growth, 1mm deep
33	H-53	CH 53D	Line	A : PDA press-plate	Vertical hull interior, 103-107	Oily surface under window, heavy growth, 1mm deep
34	H-53	CH 53D	Line	J : Dry swab in centrifuge tube	Vertical hull interior, 103-107	Oily surface under window, heavy growth, 1mm deep
35	H-53	CH 53D	Line	J : Dry swab in centrifuge tube	Around window bolts	Fuzzy black growth
36	H-53	CH 53D	Line	K : Paint chip	Tail section	
37	H-53	CH 53D	Line	J : Dry swab in centrifuge tube	Below window	
38	H-53	CH 53D	Line	F : Dry swab, in ADS, 1.5 cm diam	Below window	
39	H-53	CH 53D	Line	K : Strip of nylon webbing	Port-side hull	
40	H-53	CH 53D	Line	K : Caulking, painted	Around window	
41	H-53	CH 53D	Line	K : Duct tape		

* Sample number corresponds to isolate number.

** Abbreviations: PDA: Potato Dextrose Agar; NA: Nutrient Agar; ADS: Anaerobic Dilution Solution. (Alpha prefix is for data sorting purposes.)

Table 2: MICKIT[®] Results

Type	#8	#9	#17	#29	#38
Sulfate Reducing (SRB)	+	+	+	+	+
Acid Producing	0	0	0	0	0
Anaerobes/facultative	0	0	++	++	+
General Aerobes	++	++	0	0	++

0 = no growth; + = weak growth; ++ = strong growth

#8, 9, 17 = salvage storage

#29 = on line awaiting depot overhaul

#38 = in hangar, not cleaned

Table 3: Fungal Isolates

Genus	Sample number	General culture appearance
<i>Pestolotia</i>	12	Dry looking white mat
<i>Trichoderma</i>	23A	Cottony white mat
<i>Epicoccum</i>	23B	Yellow-orange-pink mat
<i>Phoma</i>	24, 23AP	Dusty gray, white fringe
<i>Epicoccum</i>	23B	Yellow-orange-pink mat
<i>Aureobasidium</i>	25A	Black, oily sheen
<i>Stemphylium</i>	25B	Gray-black
<i>Penicillium</i>	27	Dark green, dry, globules of yellow exudate
<i>Hormodendrum</i>	36	Green-gray, white fringe, dry

Table 4. Gross fungal colonization after 30 days exposure as a function of coating and treatment.

Isolate	Polyurethane				Lacquer			
	alc. only	oil	lanolin	(Score)	alc. only	oil	lanolin	(Score)
23A	3	2	2	7	4	2	2	8
25A	2	2	2	6	3	2	3	8
25B	2	2	2	6	2	2	2	6
27	3	3	2	8	3	3	3	9
36	4	4	4	12	5	5	5	15
(Score)	14	13	12	39	17	14	15	46

Scale of 1 to 5: 1= Little to no growth: not substantially different from day 1;
5 = Very heavy growth: entire surface covered

Table 5: Industrial biocide data† for fungicidal compounds with no listed human hazards. (Mold, yeast, and stain fungi)

	Product Name	Mold, yeast	Stain fungi	Bact	Toxicological Data (acute) LD50's *	Biodegradability	Active Ingredient(s)	Form	Chemical Class
1	Aquacar 542	•		•	none	none	Glutaraldehyde+quaternary ammonium salt	Aq. sol'n	Aldehydes, Quat. Amm. Cmpds
2	Aquacar 545	•		•	none	none	Glutaraldehyde	Aq. sol'n	Aldehydes
3	Cinon AF	•			Rat (oral) >2,000 mg/kg	none	Heterocyclic substances	Non-Aq. liquid	Unknown
4	Dantogard	•		•	Rat (oral) 2.5 g/kg (more)	none	ethyl & methyl hydantoins	Aq. sol	Imidazole
5	Dantogard Plus	•		•	none	none	ethyl hydantoin & carbamate	Powder	Imidazole & carbamate
6	Degussa Formac 40	•		•	Rat 2.5 g/kg	none	Acrolein polymer & formaldehyde	none	Aldehydes
7	Densil ND	•			none	none	none	Aq. dispersion	Unknown
8	Densil P	•		•	none	none	Dithio-2,2'-bis(benzomethyl)-amid	Aq. dispersion	Thioamide
9	Densil S	•			none	none	...Tetrachloro-4(methyl sulphonyl) pyridine	Aq. dispersion	Pyridine
10	Integra 22	•	•		Rat (oral) 2.57 g/kg (more)	none	... hydroxymethyl urea	Aq. solution	Imidazole, urea
11	Integra 44	•	•		Rat (oral) 1 g/kg (more)	none	Na hydroxymethylglycinate	Aq. solution	Other
12	Mergal BCM	•	•		Rat (oral) >16 g/kg	none	benzimidazole derivative	Powder	Imidazole
13	Nuodex Copper 8%	•			Rat (oral) 6.5 g/kg	none	copper naphthenate	Non-aq. liquid	Copper
14	Nuodex Zinc 8%	•			Rat (oral) 5 g/kg	none	zinc naphthenate	Liquid	Zinc
15	PMDS 10	•			none	none	phenyl mercury dodecanyl succinate	Non-aq. liquid	Mercury
16	PMO	•		•	none	none	phenyl mercury oleate	Non-aq. liquid	Mercury
17	Piror 842	•		•	none	none	Glutaraldehyde+quaternary ammonium salt	Aq. sol'n	Aldehydes, Quat. Amm. Cmpds
18	Piror P-704 & P-72	•		•	none	none	halo & methyl propanediol & isothiazolins	Liquid	Isothiazolinone, nitroparaffin
19	Preventol A8	•			none	yes (see Guide)	chloro & methyl thiazole-ethanols	Powder	Alcohol, triazole
20	Promexal W50	•		•	none	none	methylated isothiazolin	Aq. sol'n	Isothiazolinone
21	Promitent	•		•	Rat (oral) 4.3 g/kg	none	acid amide+isothiazolinone	Liquid	Amide, isothiazolinone
22	Prosan S-10	•		•	none	none	sodium dimethyl & ethylene dicarbamate	liquid	Thiocarbamate
23	Proxel BD & BD20	•		•	none	yes (see Guide)	1,2-benzisothiazolin-3-one	Aq. dispersion	Isothiazolinone
24	Proxel GXL	•		•	none	yes (see Guide)	1,2-benzisothiazolin-3-one	Liquid sol'n	Isothiazolinone

† From "Guide to Industrial Biocides", Paint Research Association, Smith and Springle, 1995

* Note that "none" means that no data was available for that chemical or that it was simply left off; "more" indicates that further toxicological data was listed in the Guide for other organisms.

Figure 1: Electrochemical Test Cell for Fungus-induced Corrosion in Sub-aerial Conditions

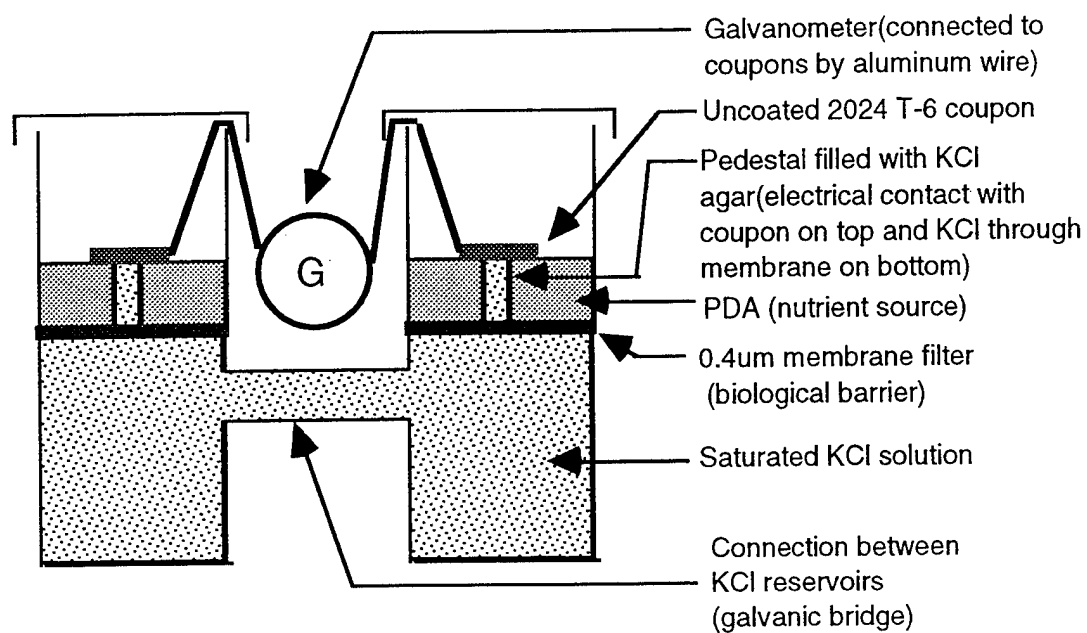
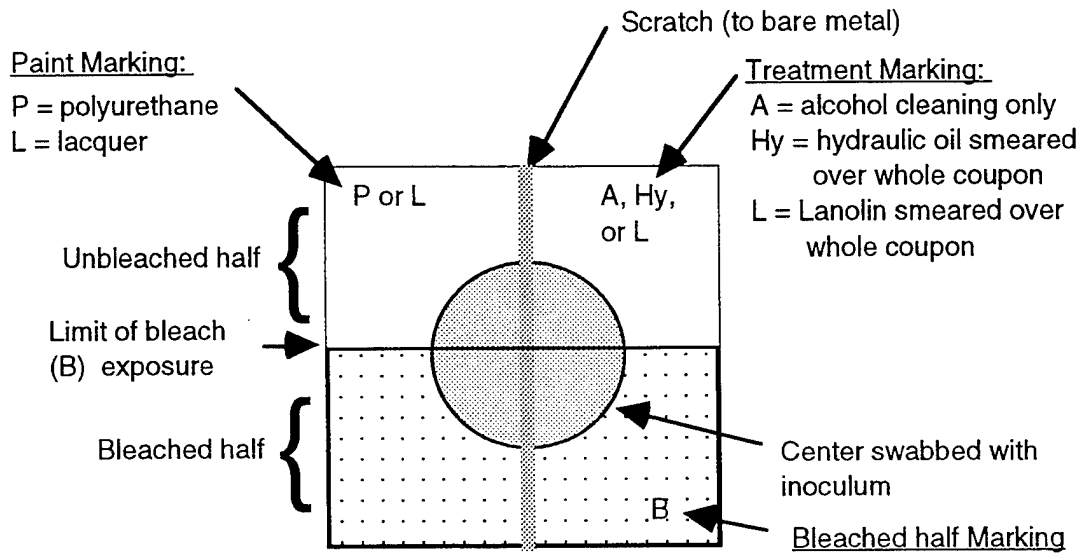


Figure 2: Experimental Scheme for Aggressiveness Tests on 1 x 1 inch Alloy Coupons



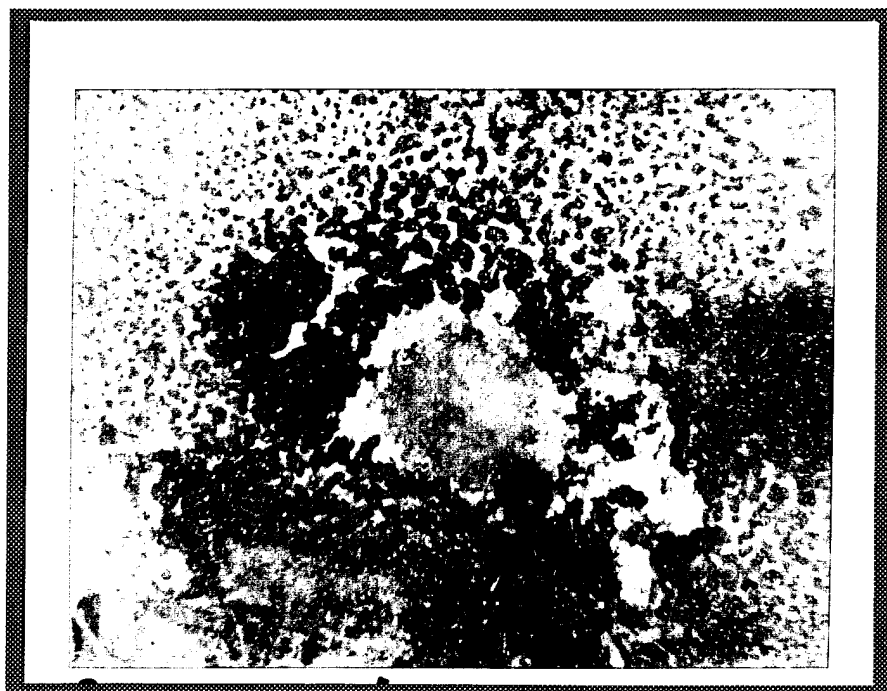


Figure 3: Oily type of fungus found on H-53 polyurethane coated interior surfaces. Note paint was removed when center of mass was removed (painted fiberglass) (6x).

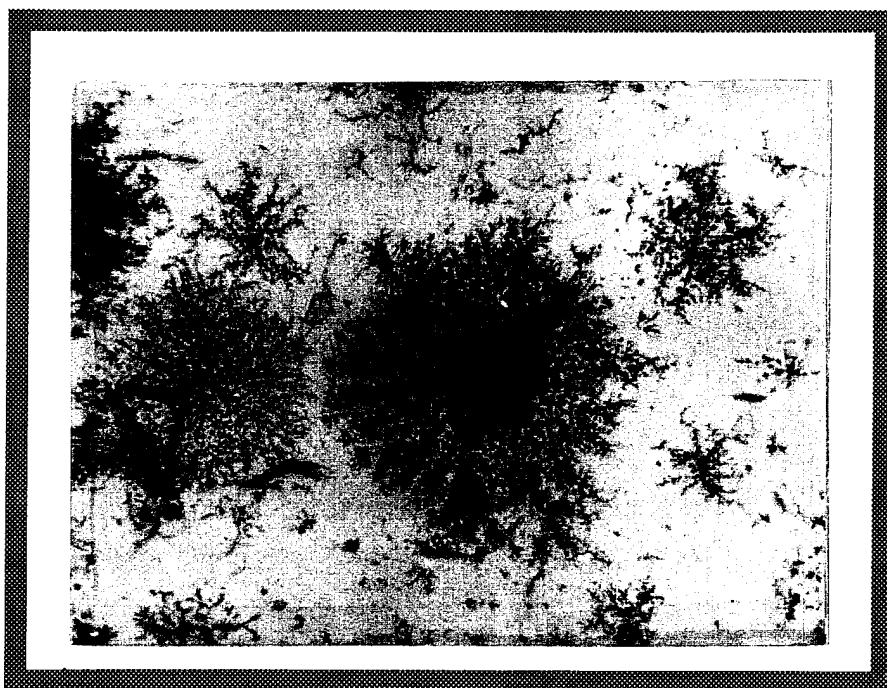


Figure 4: Dry type fungal growth on polyurethane coated aluminum (6x).

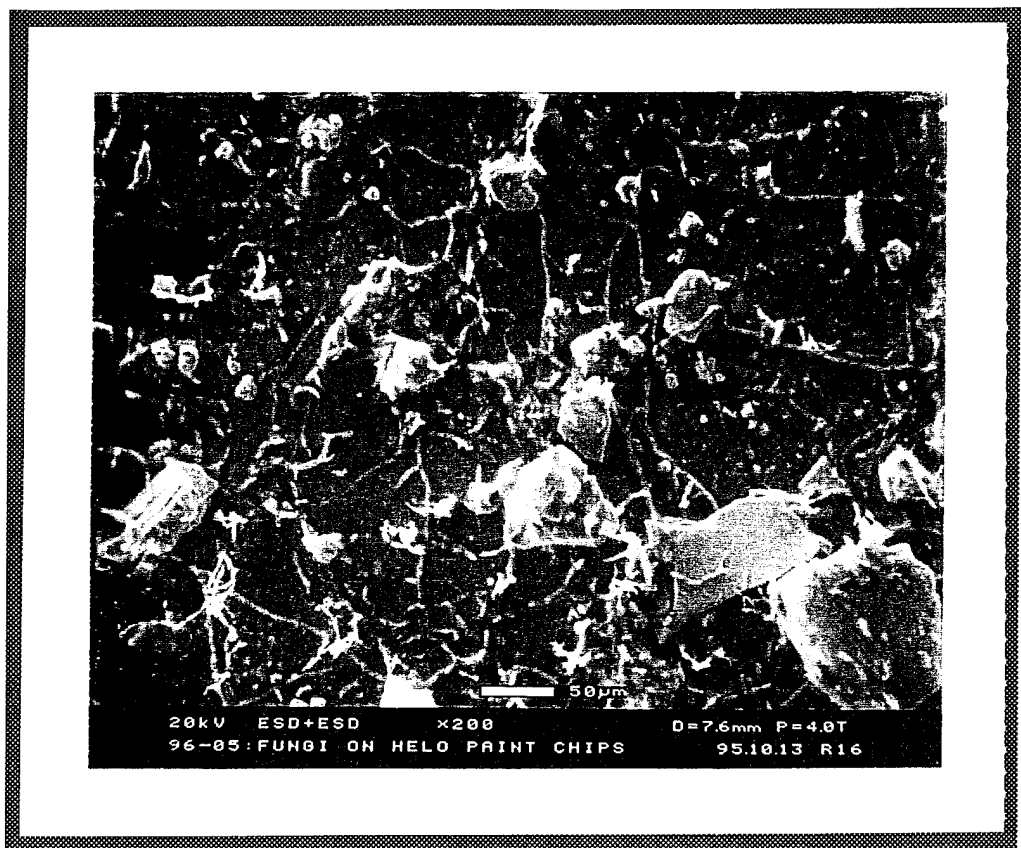


Figure 5a: Fungi and debris on polyurethane paint on interior of H-53 (200x).

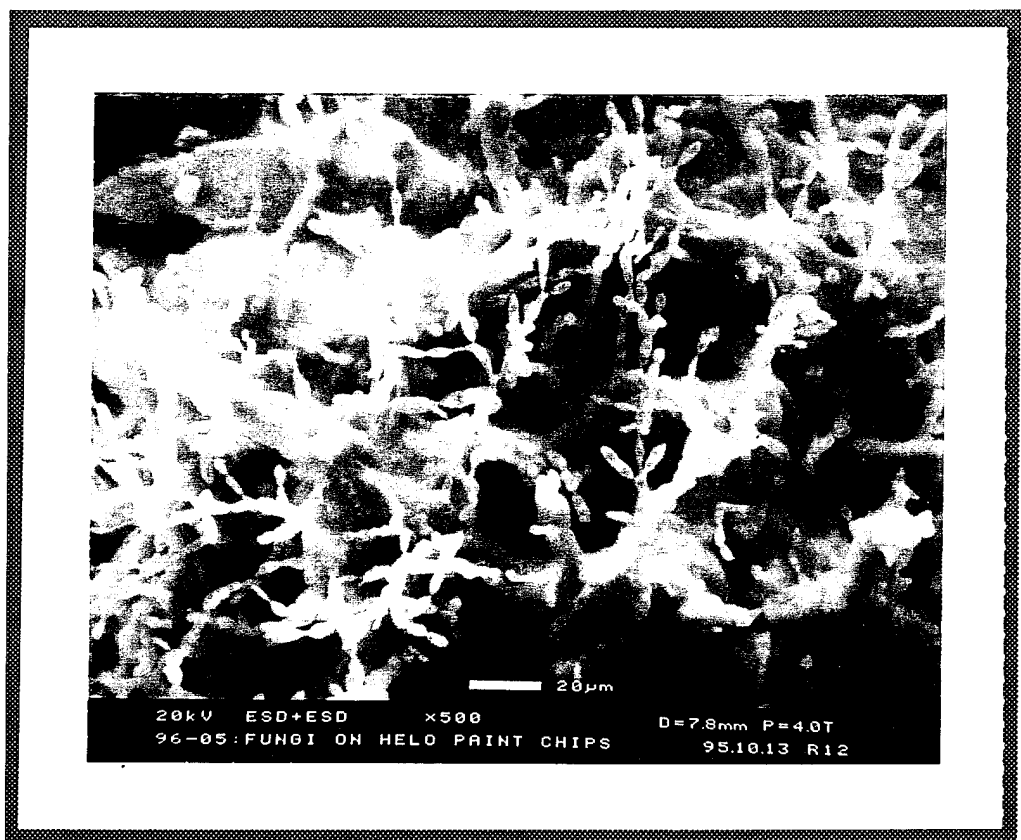


Figure 5b: Fungal mass on polyurethane paint surface (500x).

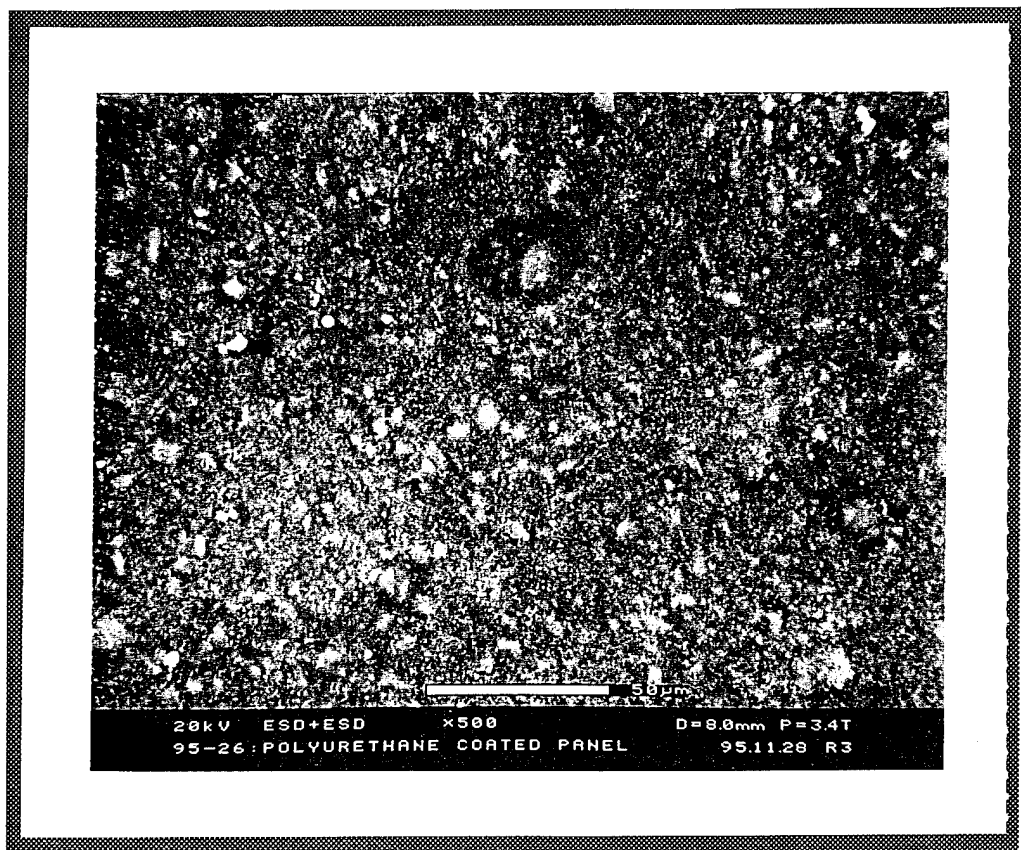


Figure 6a: Polyurethane paint surface before exposure (500x).

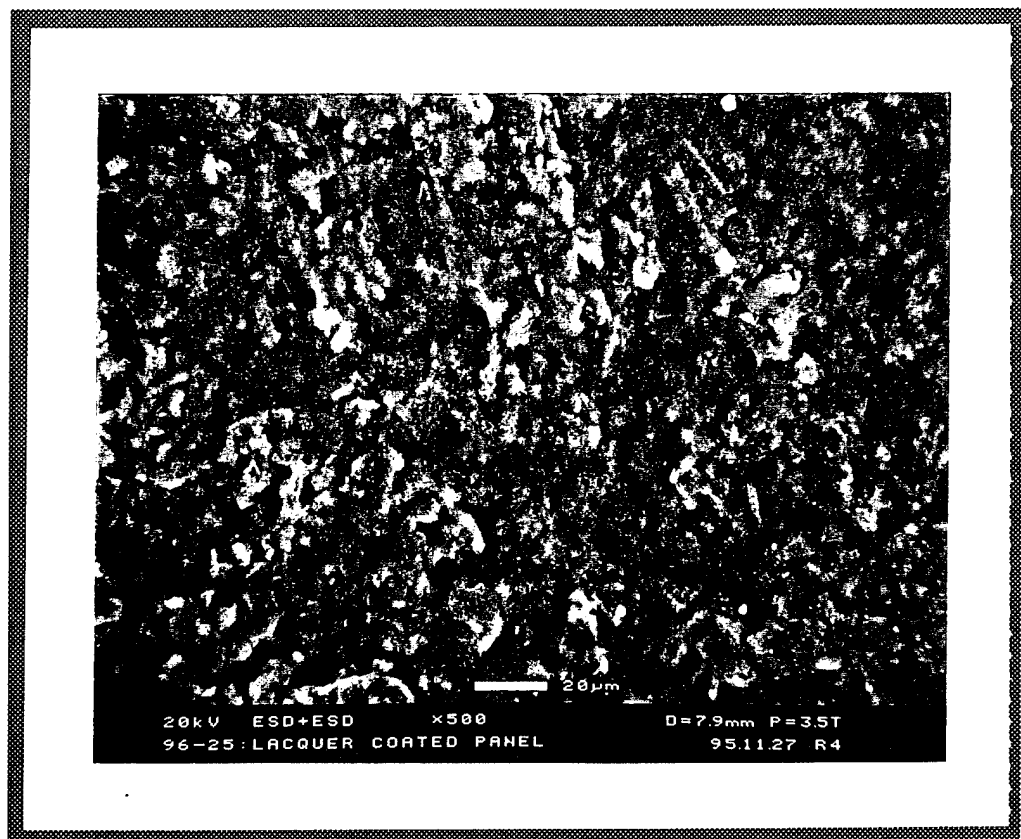


Figure 6b: Lacquer paint surface before exposure (500x).

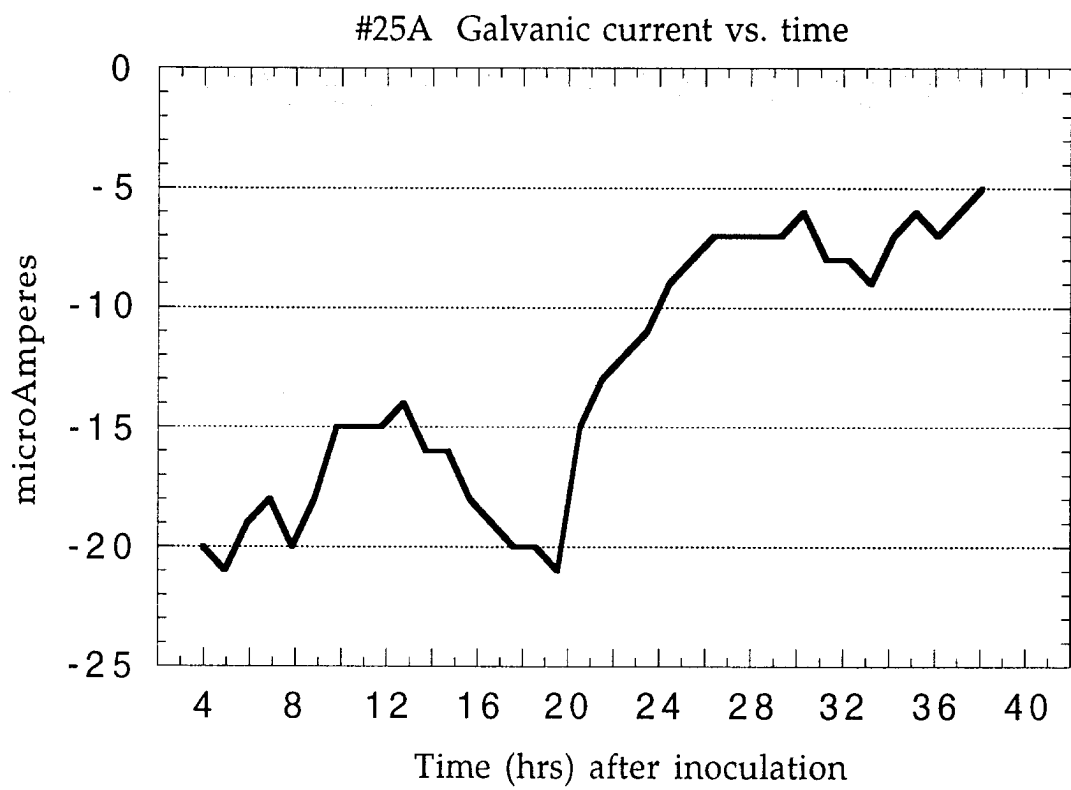
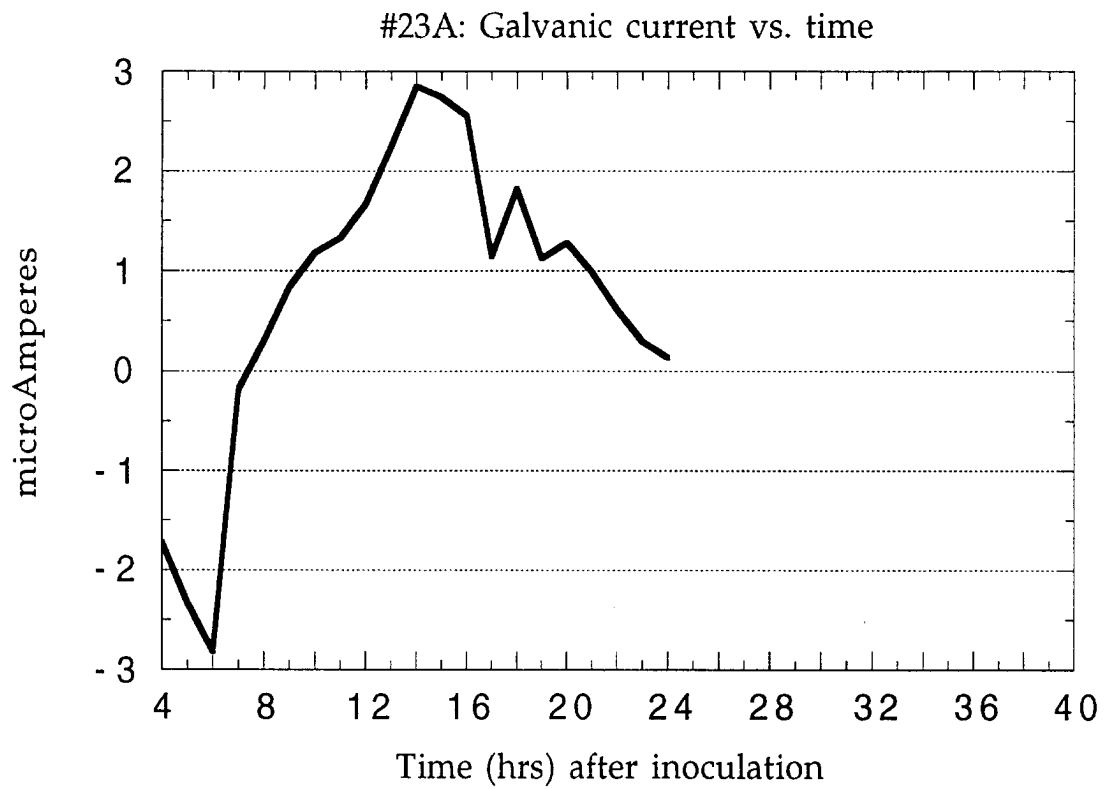


Figure 7: Galvanic currents in 2024 aluminum alloy in electrochemical test cells.



Figure 8: Mycelial mass on polyurethane coated coupon after 30 days (250x).

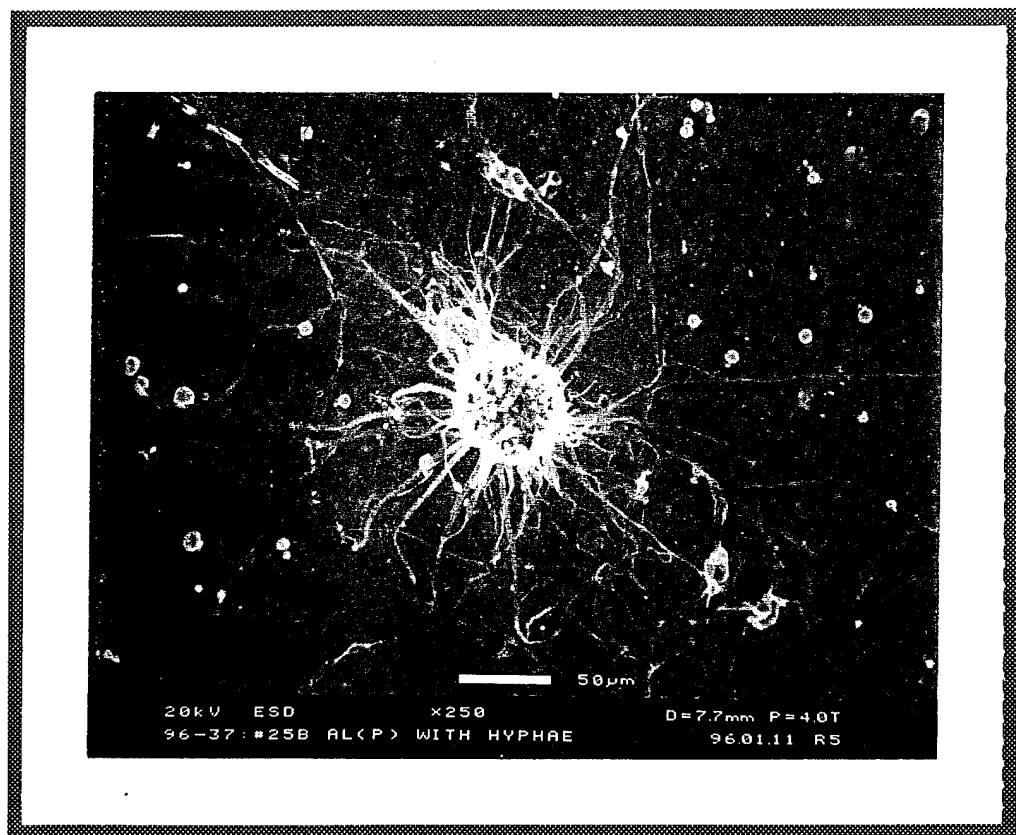


Figure 9: Germinating mycelia on polyurethane coated coupon after 30 days (250x).

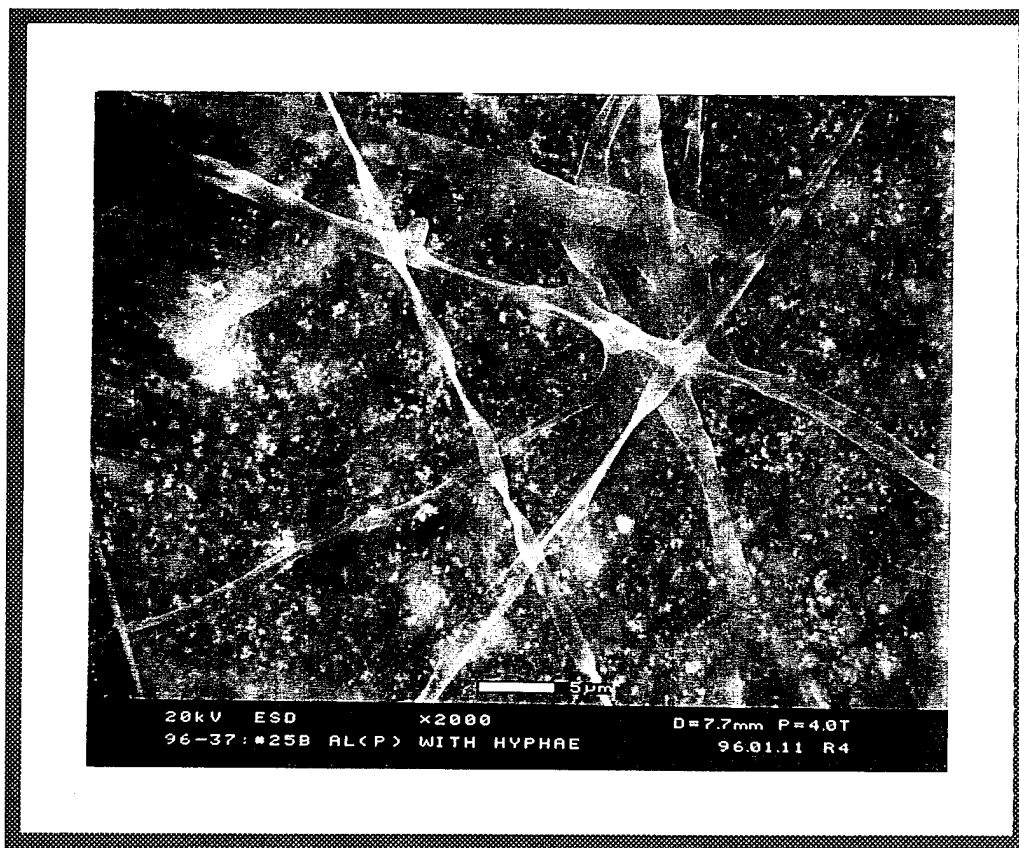


Figure 10: Hyphae attached to polyurethane coated coupon (2000x).

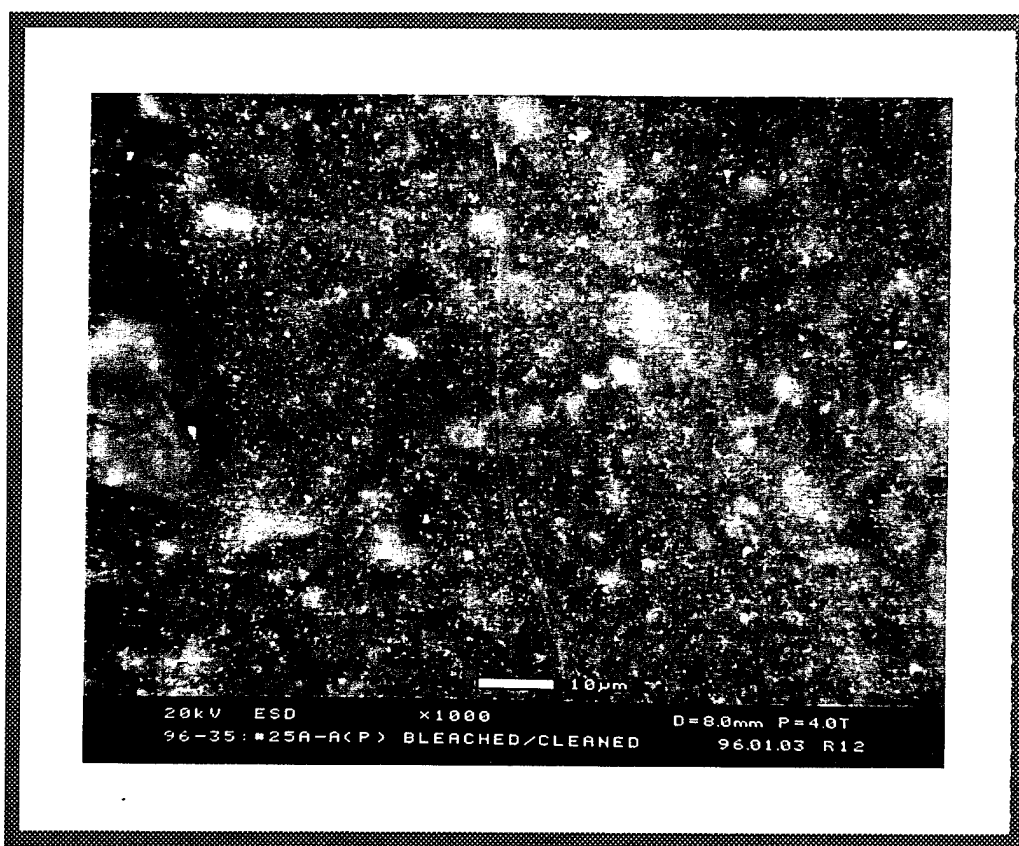


Figure 11: Polyurethane coated coupon surface after alcohol cleaning (1000x).

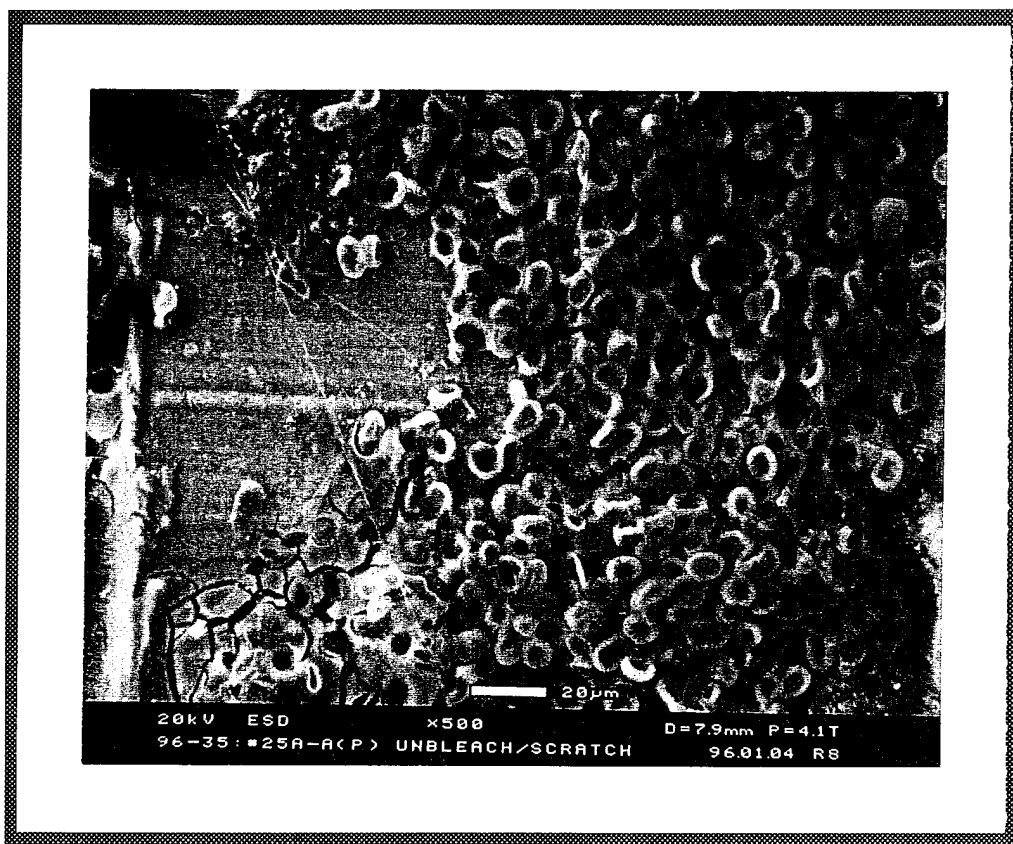


Figure 12: Fungal spores in scratch exposing bare metal, unbleached portion. Note hyphae at 11 o'clock and corrosion at 7 o'clock. (500x)

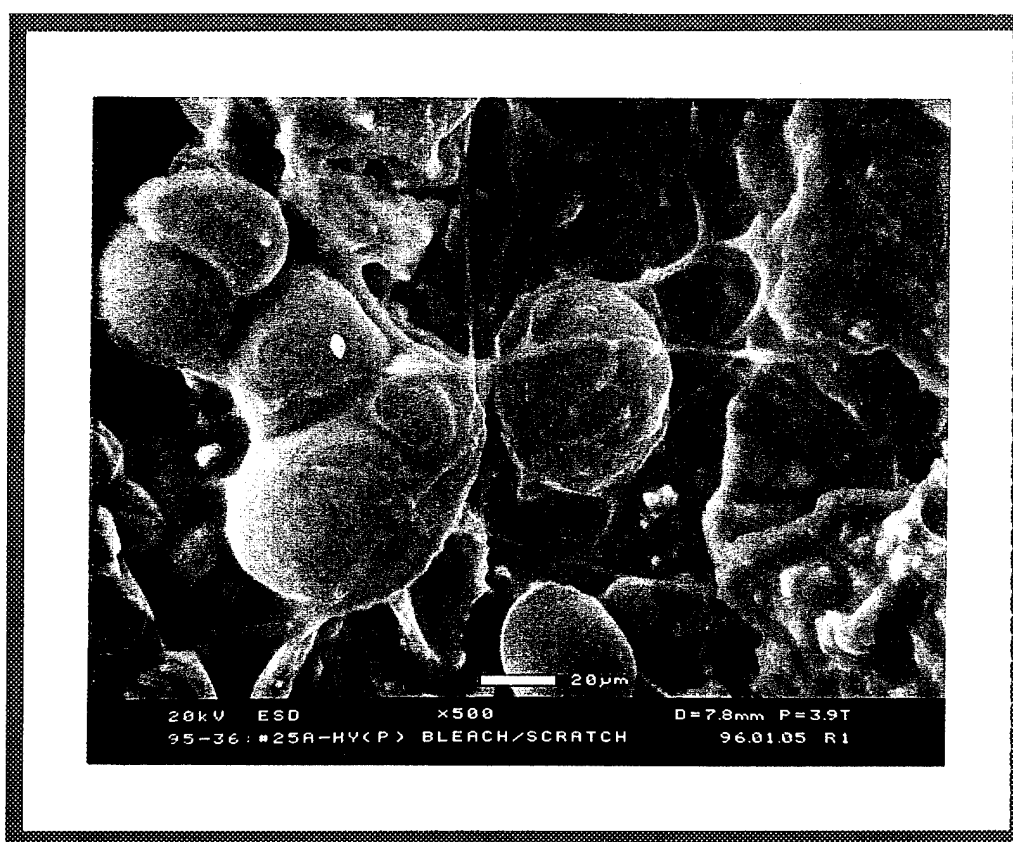


Figure 13: Hyphae on corrosion product in bleached portion of scratch (500x).

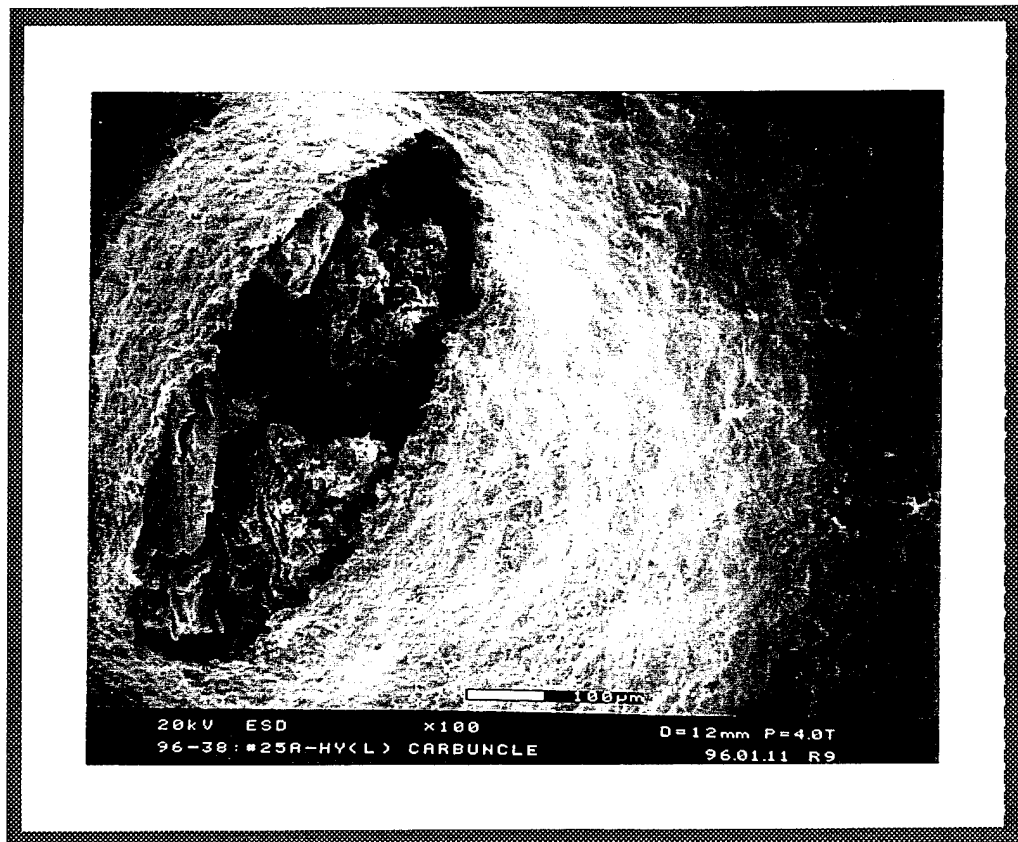


Figure 14: Blister with corrosion on lacquer paint (100x).

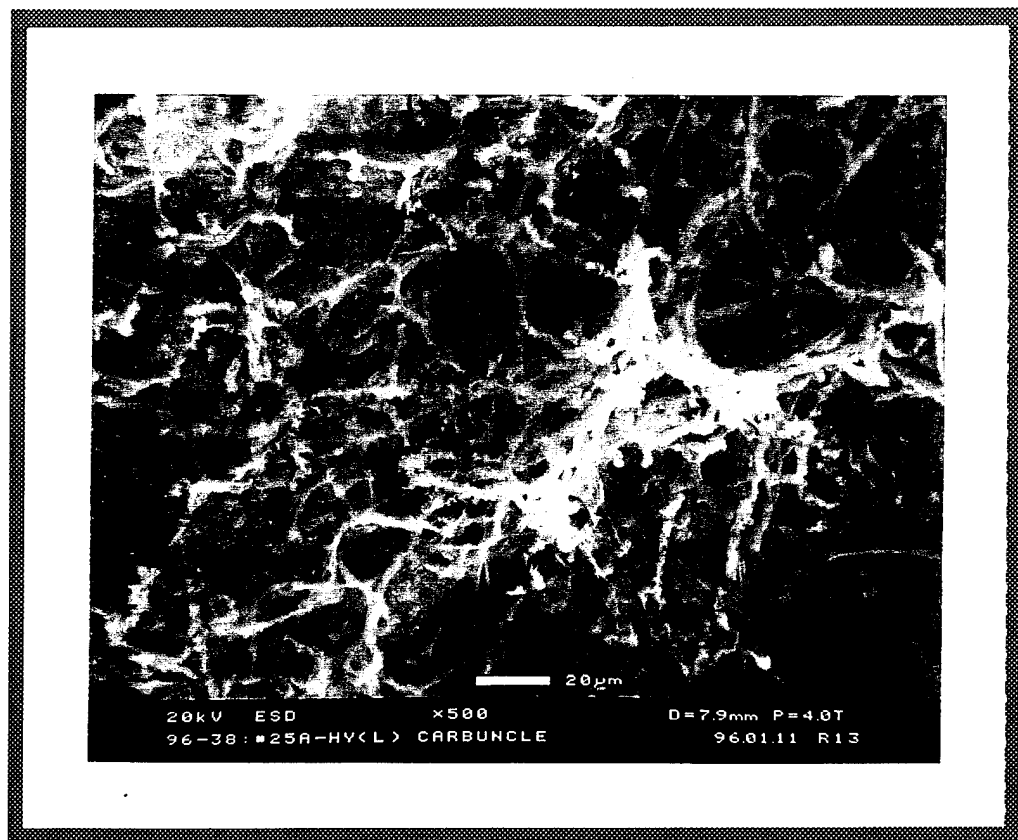


Figure 15: Fungal mycelia covering sides of blister in Fig. 14 (500x).

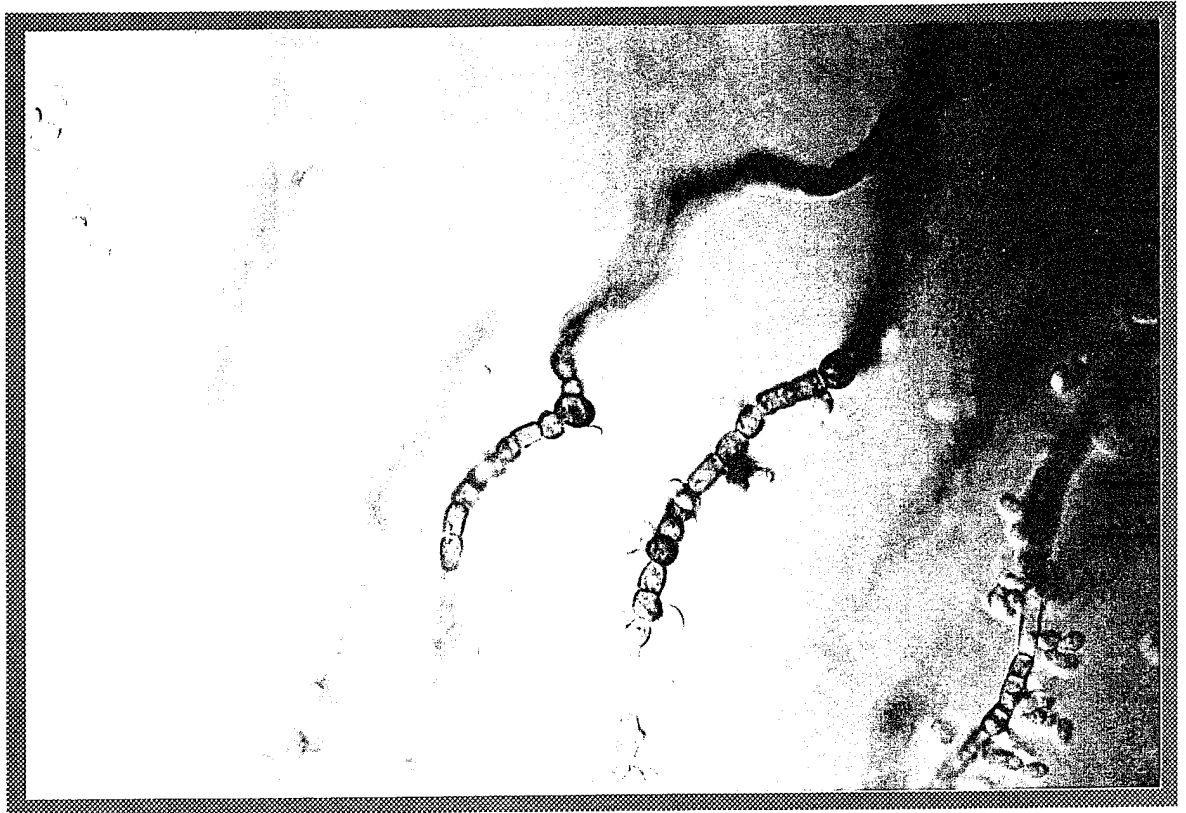


FIGURE A1: *Aureobasidium*.



FIGURE A2: *Hormodendrum*.

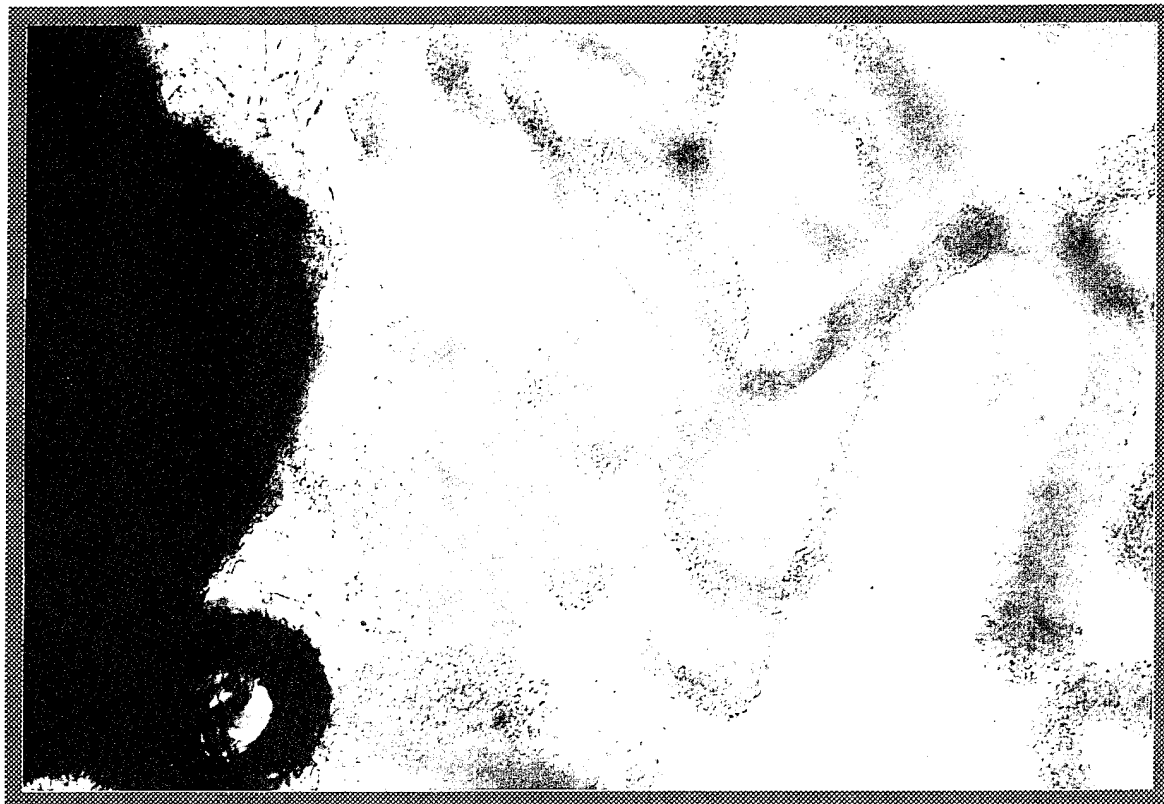


FIGURE A3: *Phoma*.

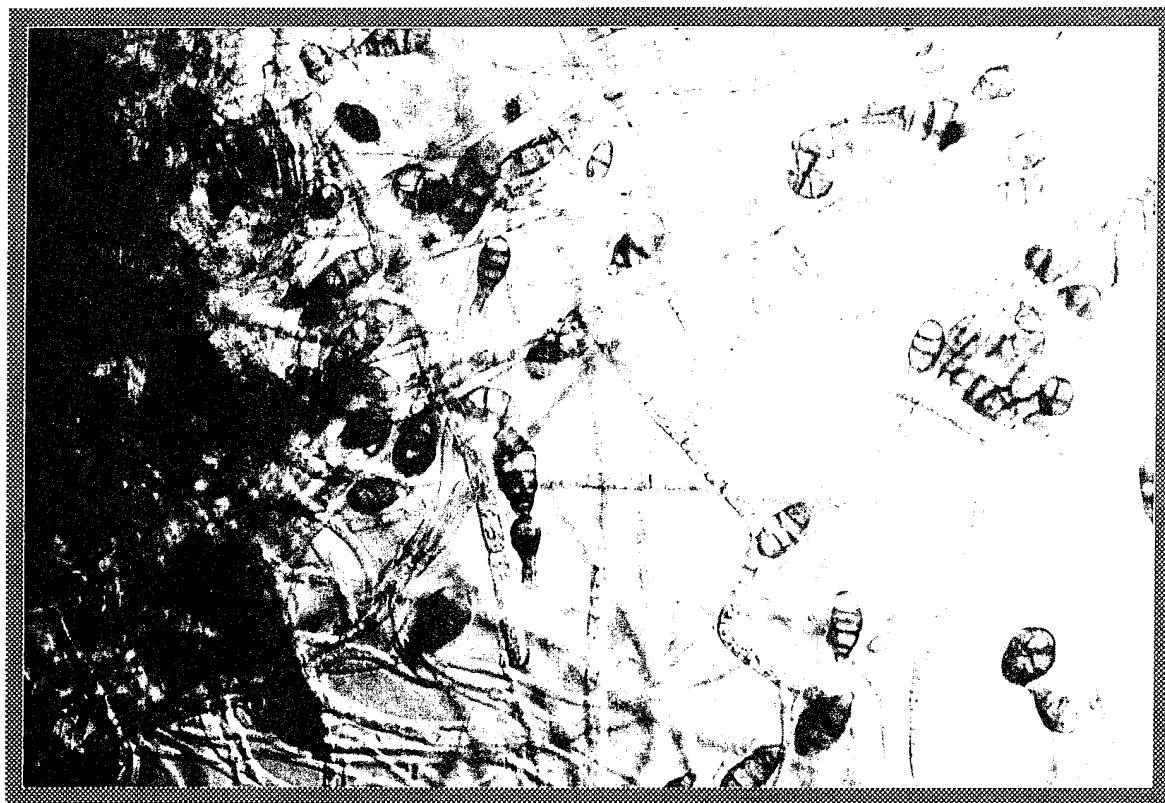


FIGURE A4: *Alternaria*.

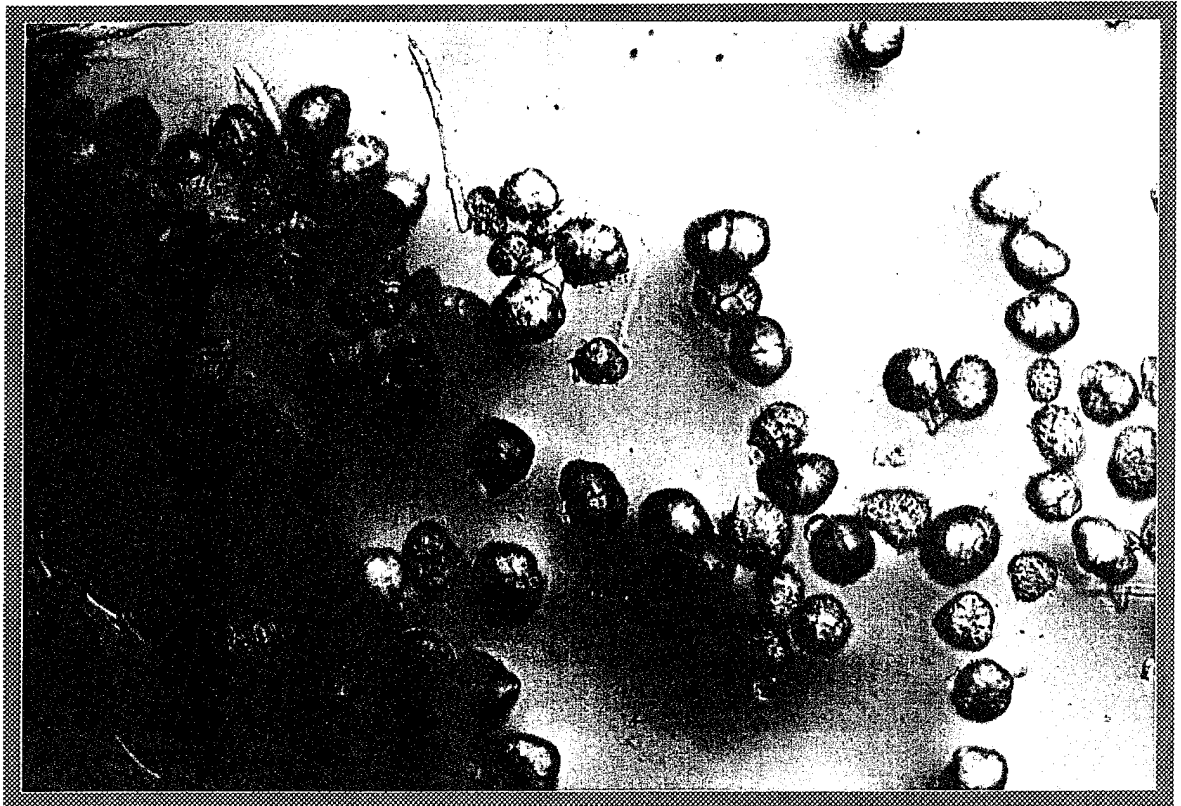


FIGURE A5: *Epicoccum*.

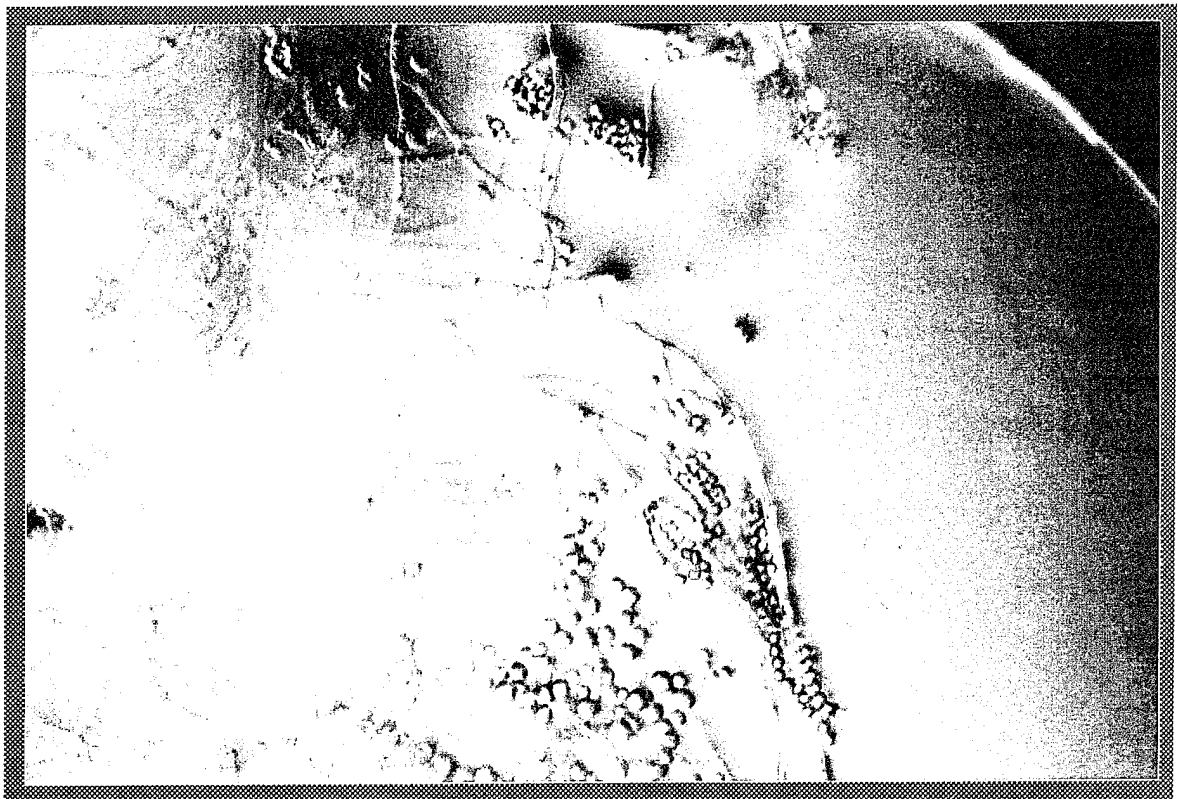


FIGURE A6: *Penicillium*.

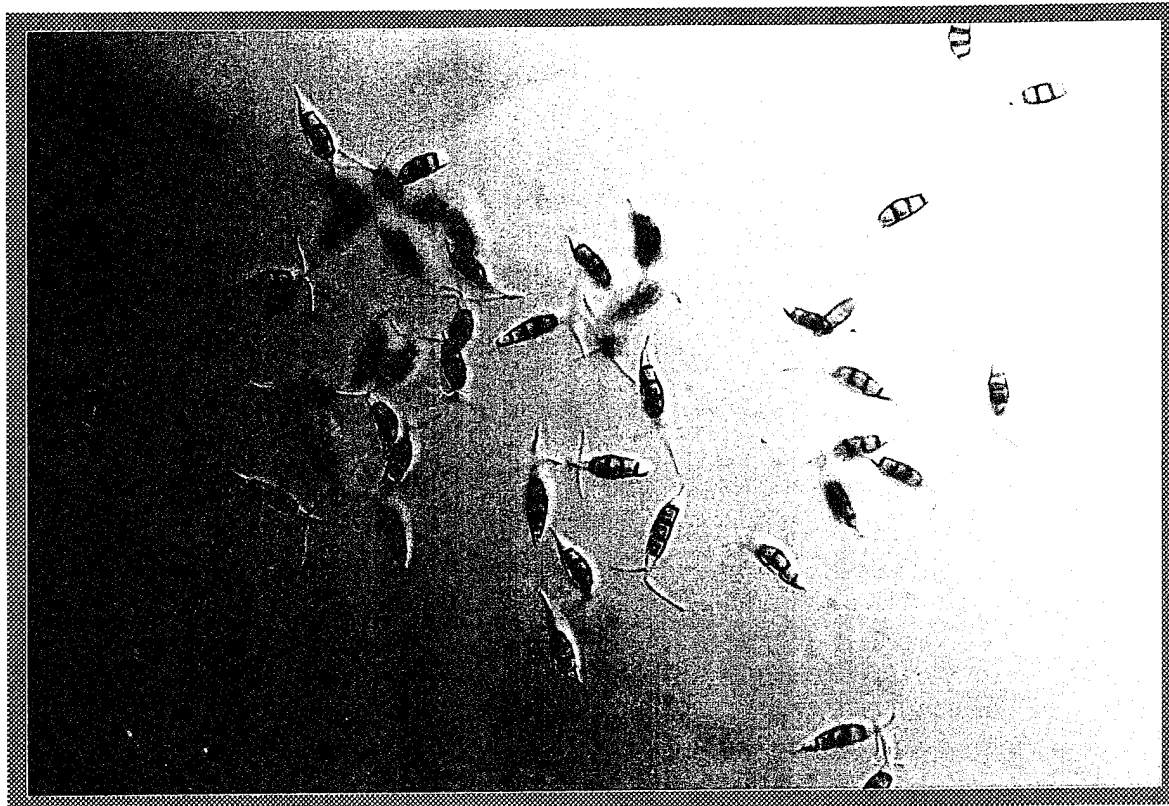


FIGURE A7: *Pestalotia*.



FIGURE A8: *Stemphylium*.